DISSERTATION

FOR THE AWARD OF DEGREE

MASTER OF SCIENCE

SESSION 2021-2024

# ASSOCIATION OF THE -866G/A POLYMORPHISM IN THE UCP2 GENE WITH TYPE 2 DIABETES MELLITUS

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## ***ACKNOWLEDGEMENT***

*Thanks to the almighty with whose blessings, this Voyage has come to its final halt. The eye's look ahead & the heart returns back, in the fond remembrance of all these gentle breezes, that helped this boat sail down to its destination. Realization of this goal would remain a distinct dream, but for the scholastic guidance of my teachers and the unstained support of colleagues and my family.*

*It's my proud privilege, to express my heartfelt gratitude to my esteemed teacher and guide* ***Dr. Jyoti Batra****, Dean Research & Professor, Department Of Biochemistry, Santosh Medical College and Hospital, Ghaziabad (U.P.) who devoted her precious time to advise me on every aspect of my work. Her deep sense of involvement in my work has helped me to accomplish this study. Inspite of my best possible endeavor words cannot approximately express the honor acknowledging the immense debt that I owe to my kind hearted & learned teacher. Without her able & stream lined guidance, constant and vigilant supervision and immutable encouragement this work would not have seen the light of the day.*

*I specially wish to thank my respected co-guide* ***Dr. Ashok Kumar*** *Professor & Head of Department of Internal Medicine, Santosh Hospital Ghaziabad, for helping and guiding me throughout this study, and for allowing me to pursue this study in the Department of Internal medicine. I shall always cherish the moments when his guidance shone like a light through the darkness of my ignorance. My head bows with unfathomable indebtedness and in deep regard to his throughout the work.*

*I extend my wholehearted gratitude and special thanks to my respected HOD,* ***Dr. Poonam Aggarwal*** *Professor & HOD Department of Biochemistry, Santosh Medical College and Hospital, Ghaziabad (U.P.) whose continuous support, guidance and encouragement has been a great help in my thesis work.*

*I render my thanks to* ***Dr. Shalabh Gupta****, M.S., Dean-Academics, Santosh Medical College and Hospital, Ghaziabad (U.P.) for permitting me to undertake this work.*

*I wish to express thankful to **Dr. R.N. Gupta**, Associate Professor, Department of Biochemistry, Santosh Medical College and Hospital, Ghaziabad (U.P.), **Mr. Sunil Shah**, Senior Tutor, Department of Biochemistry, Santosh Medical College and Hospital, Ghaziabad (U.P.) who always stood as aguidance in the path of success for me.*

*I also wish to thank the technical staff of the Department of Biochemistry, specially **Mr. Ashok Kaushik** and **Mr. Prabhat Kumar** and technical staff of Central Research Facility, **Ms. Anmol, Ms. Tanu, Mr. Shabin** and **Ms. Leena** for extending all possible help.*

*I am also thankful to my friends & colleague **Seenu**, **Gaurav Saini**, **Apoorva, Gaurav Maurya, Jyotsana** and **Ahana** and for their timely help and support.*

*My parents Late **Mr. Sandeep Kumar** and **Mrs. Anupama Vishnoi**, deserve a very special mention for their extraordinary blessings, unparalleled affection, patience, and support and their belief in all my endeavors and also would like to thank my sister **Himanshi** my brother **Vansh** and my brother in law **Vishal** for their encouragement and support.*

*I would be failing in my duties if I don't pay my special sense of obligation to all of Patients who despite being in so much pain and agony were always eager to co-operate more and more. I wish them a speedy recovery and healthier life.*

# ABBREVIATIONS

| Sr No | Abbreviation | Full form |
|---|---|---|
| 1. | ADA | American Diabetes Association |
| 2. | ATP | Adenosine triphosphate |
| 3. | DM | Diabetes Mellitus |
| 4. | T1DM | Type 1 Diabetes Mellitus |
| 5. | T2DM | Type 2 Diabetes Mellitus |
| 6. | IDF | International Diabetes Federation |
| 7. | UCP | Uncoupling Protein |
| 8. | UCP2 | Uncoupling Protein 2 |
| 9. | SNP | Single Nucleotide Polymorphism |
| 10. | TG | Triglycerides |
| 11. | LDL | Low Density Lipoprotein |
| 12. | VLDL | Very Low Density Lipoprotein |
| 13. | HDL | High Density Lipoprotein |
| 14. | ROS | Reactive Oxygen Species |
| 15. | WHO | World Health Organisation |
| 16. | GD | Gestational Diabetes |
| 17. | IR | Insulin Resistance |
| 18. | HLA | Human Leukocyte Antigen |
| 19. | DEP | Diabetes of Exocrine Pancreas |
| 20. | FBG | Fasting Blood Glucose |
| 21. | EDTA | Ethylene Diamine Tetra Acetic Acid |

| | | |
|---|---|---|
| 22. | DNA | Deoxyribonucleic acid |
| 23. | PCR | Polymerase Chain Reaction |
| 24. | sec | seconds |
| 25. | OPD | Out Patient Department |
| 26. | HbA1c | Glycated heamoglobin |
| 27. | GSIS | Glucose- stimulated insulin secretion |
| 28. | g | Gram |
| 29.. | mRNA | messenger RNA |
| 30. | RFLP | Restriction fragment length polymorphism |
| 31. | TAE | Tris acetate EDTA |
| 32. | TBE | Tris borate EDTA |
| 33. | EtBr | Ethidium Bromide |
| 34. | dNTPs | Deoxynucleotide triphosphates |
| 35. | µl | microliter |
| 36. | BAT | Brown adipose tissue |
| 37. | ARMS-PCR | Amplification refractory mutation system polymerase chain reaction |

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# INTRODUCTION

## 1. Background

Diabetes Mellitus has become a major public health concern due to its high prevalence worldwide. A major cause of mortality and morbidity is complications from diabetes mellitus. Rapid socioeconomic development as well as demographic changes has resulted in an explosive rise in diabetes mellitus prevalence in India over the last four decades (Unnikrishnan et al. 2016). The International Diabetes Federation (IDF) projects that 463 million persons (aged 20 to 79) would receive a diabetes diagnosis in 2019, increasing to 578 million by 2030, and 700 million globally by 2045. The prevalence is notably higher in urban areas as opposed to rural regions, and similarly, it is markedly elevated in high-income countries when contrasted with low-income ones (Saeedi et al. 2019).

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia (elevated blood glucose levels) due to either insufficient insulin production, ineffective use of insulin, or a combination of both (Punthakee at al. 2018). The detrimental impacts of hyperglycemia are divided into two main categories: macrovascular and microvascular complications. Microvascular complications encompass retinopathy, diabetic nephropathy, and neuropathy, while macrovascular complications consist of stroke, coronary artery disease, and peripheral arterial disease (Halim and Halim 2019). Pancreatic beta cells (β-cells) are responsible for secreting insulin, a hormone crucial for enabling glucose absorption into cells to generate energy. Additionally, insulin plays a role in various other physiological functions (Tan et al. 2019).

Diabetes Mellitus can be classified into several main types. Type 1 diabetes occurs when pancreatic beta cells are destroyed, resulting in a lack of insulin production and absolute insulin deficiency. Type 2 diabetes develops due to a combination of insulin resistance and a progressive decline in insulin secretion. Gestational diabetes mellitus (GDM) is diagnosed during pregnancy's later stages and doesn't fit the criteria for overt diabetes. Also, the conventional understanding that type 2 diabetes exclusively affects adults and type 1 diabetes solely impacts children is outdated, given that both conditions can occur across all age groups (American Diabetes Association, 2020). .World Health Organization has reported that the 9% of the total world population is diabetic out of which 90% cases are of Type 2 Diabetes Mellitus (World Health Organization, 2016)

### 1.1 Type 2 Diabetes Mellitus

Type 2 Diabetes Mellitus (T2DM) is a prevalent metabolic condition globally, largely brought about by two key factors: pancreatic β-cells not producing enough insulin, and insulin-sensitive tissues not reacting properly to insulin (Roden and Shulman, 2019). Since insulin plays a crucial role in maintaining balanced glucose levels, the molecular pathways responsible for insulin production, release, and detection are meticulously controlled. Any disruptions in these pathways can cause metabolic dysregulation, contributing to the onset of the disease (Galicia-Garcia et al. 2020). As the disease progresses, insufficient insulin secretion fails to regulate blood sugar levels, resulting in hyperglycemia. Individuals with Type 2 Diabetes Mellitus (T2DM) typically exhibit obesity or higher levels of body fat, primarily concentrated around the abdomen. In this state, adipose tissue contributes to insulin resistance (IR) through various inflammatory pathways, including increased release of free fatty acids (FFAs) and dysregulation of adipokines. The escalating rates of obesity, sedentary lifestyles, high-calorie diets, and aging populations are

the primary drivers behind the T2DM epidemic, leading to a fourfold increase in both its incidence and prevalence (Chatterjee et al. 2017; Zhou et al. 2016). T2DM is a progressive disorder that causes poor glycemic control over time. It increases the risk of many metabolic problems. The indication is a rise in HbA1c, which can severely affect their prognosis due to treatment delays (Holman et al. 2008).

### 1.2 Uncoupling Proteins (UCPs)

The mitochondria are pivotal in cell metabolism, the production of reactive oxygen species (ROS), and programmed cell death, facilitated by carrier proteins situated in the inner mitochondrial membrane (Roy et al. 2008). UCPs are the transmembrane proteins which are present in the inner mitochondrial membrane and they are members of the SLC25 mitochondrial anionic transporter family. The mouse and human genomes both contain the five-membered UCP family (UCP1-UCP5), each UCP is numbered in the order in which they were discovered. These ion-carrier proteins carry protons (H+) to the mitochondrial matrix, where they release the proton motive force as heat and uncouple the oxidation of the substrate from the synthesis of ATP (Demine et al. 2019). The expression patterns of uncoupling proteins (UCPs) vary across different tissues in the human body. UCP1 is mainly found in BAT. UCP2 exhibits expression in multiple tissues including skeletal muscles, heart, liver, kidney, lung, pancreas, spleen, and macrophages. UCP3 is primarily expressed in brown adipose tissue and muscles. UCPs 4 and 5 have specific neuronal expression and are mainly found in brain tissues (Echtay at al. 2018). UCPs 2, 4, and 5 are termed neuronal UCPs due to their predominant localization within the central nervous system (CNS) (Hoang et al. 2015).

### 1.3 Uncoupling Protein 2 And Type 2 Diabetes Mellitus

Uncoupling protein 2 (UCP2) is expressed in a various tissues and plays roles in oxidative stress protection, negative control of insulin production by beta cells, and fatty acid metabolism. All of these mechanisms are linked to the etiology of DM2 and its chronic consequence (Souza et al. 2011). UCP2 dissipates the proton gradient created across the mitochondrial inner membrane, uncoupling oxidative phosphorylation from ATP synthesis and decreasing ATP production (Dieter et al. 2020). This results in decreased KATP channel inactivation and decreased insulin secretion (Cadenas et al 2018). These mechanisms are directly linked to the onset of Type 2 Diabetes Mellitus.

Under hyperglycemic conditions, UCP2 expression was increased in human pancreatic β-cells. In vitro and in rodent models under hyperglycemic and hyperlipidemic circumstances, β-cell activity is improved by UCP2 genetic deficit (Gomathi et al. 2019). UCP2 knockout mice have been shown to have hypoglycemia due to increased glucose stimulated insulin secretion and higher ATP levels in isolated islets (González-Barroso et al. 2008). Hyperinsulinemia and hypoglycemia were seen in UCP2 knockout mice, indicating that UCP2 is involved in the secretion of insulin and the metabolism of glucose (Sreedhar and Zhao, 2017).

Single nucleotide variation in the UCP2 promoter region has been linked to obesity, hypertension, cardiovascular disease, and diabetes. A polymorphism in the UCP2 promoter region acts to increase UCP2 expression which leads to reduce insulin secretion, resulting in early-onset Type 2 Diabetes Mellitus. The -866G/A polymorphism in the UCP2 promoter is linked to differences in UCP2 expression and higher levels of oxidative stress markers (Hou et al. 2020). The A allele of the UCP2 (-866) promoter region leads to increased UCP2 mRNA expression when compared to G allele. While the G allele of the UCP2 (-866) promoter region associated with reduced level of mRNA expression, which is associated with an increased risk of

obesity Individuals with AA genotype in the promoter region of UCP2 (-866) was associated with insulin resistance and Type 2 Diabetes Mellitus and there is an association between A-allele of UCP2(G-866A) polymorphism and insulin resistance (Oguzkan-Balci et al. 2013).

The -866G/A polymorphism in the uncoupling protein 2 (UCP2) gene has been investigated for its association with type 2 diabetes mellitus (T2DM) in several populations. However, the results of these investigations have been varied. A study by Gomathi et al. 2019 of 318 T2DM patients discovered that the UCP2 (G-866A) polymorphism may play a significant role in the development of T2D in the south Indian population. Another study of 850 subjects of Kashmiri north population found that the association of - 866 G/A SNP of the UCP2 gene with its expression in diabetes patients appears to be an important genetic determinant in the progression of T2DM (Din et al. 2023). However, other studies have not found a significant association between the -866G/A polymorphism and T2DM. Study in Japanese and Pima Indian populations was failed to find the association of UCP2 G-866A polymorphism with the risk of Type 2 Diabetes Mellitus Risk (Sasahara et al. 2004; Kovacs et al. 2005). A meta-analysis on European ancestry was not able to demonstrate any association between the UCP polymorphisms and T2DM (de et al. 2013).

The mixed findings of these studies suggest that the association between the -866G/A polymorphism and T2DM may be complex and may be influenced by other factors such as genetic background, environmental factors, and lifestyle factors. Further research is needed to better understand the association between the -866G/A polymorphism and T2DM in Delhi NCR population. The purpose of this study is to investigate the relationship between the -866G/A polymorphism in the Uncoupling protein 2 gene and the risk of developing Type 2 Diabetes Mellitus in the Delhi NCR region.

# AIMS AND OBJECTIVES

**AIM:** To find out the association of the -866G/A polymorphism in the Uncoupling protein 2 gene with Type 2 Diabetes Mellitus

**OBJECTIVES:**

- To investigate the association between the -866G/A polymorphism in the UCP2 gene and the risk of developing T2DM
- To determine the hyper glycaemia and .lipid profile levels.

**RESEARCH QUESTION:**

Is there an association between the -866G/A polymorphism in the UCP2 gene and type 2 diabetes mellitus?

**HYPOTHESIS:**

The -866G/A polymorphism in the UCP2 gene is associated with an increased risk of developing type 2 diabetes mellitus.

**NULL HYPOTHESIS:**

There is no association between the -866G/A polymorphism in the UCP2 gene and type 2 diabetes mellitus.

# REVIEW OF LITERATURE

Diabetes is a long-lasting health problem. It happens when your body doesn't make enough insulin which is a hormone made by the pancreas or when the insulin it makes doesn't work well. This leads to high levels of sugar in your blood, which can damage different parts of your body, like your blood vessels and nerves (Amin et al. 2018). The growing prevalence of diabetes mellitus worldwide is a pressing public health concern, creating significant challenges for individuals, caregivers, healthcare systems, and society at large (Forouhi and Wareham et al. 2019). Around 8% of children and roughly 26% of young adults worldwide are affected by diabetes mellitus (Tao et al. 2015). By 2030, according to projections from the World Health Organization, diabetes is expected to rank as the seventh leading cause of death (WHO, 2014).

## 2.1 Classification of Diabetes Mellitus

Diabetes is a varied, complex metabolic condition characterized by high blood glucose levels due to insulin resistance, inadequate insulin production, or both. Diabetes mainly categorized into Type 1 Diabetes Mellitus, Type 2 Diabetes Mellitus, and Gestational Diabetes. Type 2 diabetes (T2DM) is identified by insulin resistance and a relative insufficiency in insulin secretion. In Type 1 DM, there's typically a complete lack of beta-cell function in most cases. The autoimmune destruction of beta-cells is a common cause, although some cases are still labeled as idiopathic. Gestational diabetes mellitus (GDM) is characterized by glucose intolerance that emerges for the first time during pregnancy (Solis-Herrera et al. 2015).

## 2.2 Epidemiology of Diabetes Mellitus

The IDF Diabetes Atlas (2021) reports that roughly 537 million persons aged 20 to 79 have diabetes. The total number of individuals living with diabetes is expected to reach 643 million by

2030 and 783 million by 2045. Studies shows that 10.5% of adults aged 20 to 79 have diabetes, with nearly half unaware knowing they have the condition. According to IDF forecasts, by 2045, one in every eight persons, or roughly 783 million, will have diabetes, representing a 46% rise (Sun et al. 2022). Type 2 diabetes accounts for over 90% of cases of diabetes worldwide (Tinajero et al. 2021). Rapid urbanization and sedentary lifestyles have accelerated the rise in diabetes prevalence across many countries and areas (Meroni et al. 2021). It was estimated that in 2017, diabetes caused 5.0 million deaths among people aged 20–99. Diabetes was responsible for 9.9% of worldwide all-cause mortality among this age group. More than one-third (36.5%, or 1.8 million) of diabetes-related deaths occurred among adults under the age of 60 (Cho et al. 2018). Diabetes has progressively increased throughout the nation and around the world for the course of the past three decades, with India bearing a significant amount of the global burden. Diabetes prevalence in India has increased from 7.1% in 2009 to 8.9% by 2019. In addition, it is estimated that about 57% of adults with diabetes in India, or approximately 43.9 million, are undiagnosed. Diabetes has a mean healthcare expenditure of 92 US dollars per person, and there are 1 million deaths directly related to diabetes (Pradeepa et al. 2021). The incidence and prevalence of T2DM vary by geographical region, with more than 80% of patients residing in low- to middle-income nations, posing significant hurdles to effective treatment. Patients with T2DM have a 15% greater risk of all-cause mortality compared to people without diabetes, with cardiovascular disease (CVD) being the most common cause of morbidity and mortality linked with T2DM (Gæde et al. 2003). Table 2.1 shows a list of the top 10 countries or territories with diabetes among adults aged 20-79 years in 2021 and 2045. (IDF, 2021)

| Rank | Country | No. of people with Diabetes (millions) 2021 | No. of people with Diabetes (millions) 2045 |
|---|---|---|---|
| 1. | China | 140.9 | 174.4 |
| 2. | India | 74.2 | 124.9 |
| 3. | Pakistan | 33.0 | 62.2 |
| 4. | USA | 32.2 | 36.3 |
| 5. | Indonesia | 19.5 | 28.6 |
| 6. | Brazil | 15.7 | 23.2 |
| 7. | Mexico | 14.1 | 22.3 |
| 8. | Bangladesh | 13.1 | 21.2 |
| 9. | Japan | 11.0 | 20.0 |
| 10. | Egypt | 10.9 | 13.4 |

**Table 2.1 List of the top 10 countries or territories with diabetes among adults aged 20-79 years in 2021 and 2045**

### 2.3 Pathophysiology of Type 2 Diabetes Mellitus

Abnormal blood glucose levels arise from malfunctioning feedback loops between insulin action and secretion. This affects insulin action in insulin-sensitive tissues like liver, muscle, and adipose tissue (insulin resistance in type 2 diabetes) and insulin secretion by pancreatic islet β-cells (β-cell dysfunction in type 2 diabetes) (Zheng et al. 2018). Inadequate glucose sensing leads to beta cell malfunction and high glucose levels. Hyperglycemia occurs when glucose levels are consistently above the physiological range. Systemic insulin resistance leads to impaired insulin signaling in glucose-recipient tissues, resulting in persistent hyperglycemia. Diabetes is mostly caused by beta cell failure rather than insulin resistance. Both pathogenic states interact and

could worsen diabetes (Cerf, 2013). Type 2 diabetes (T2DM) is caused by a combination of unchangeable genetics and adjustable environmental variables. These interactions raise the likelihood of insulin resistance, beta cell malfunction, obesity, and eventually diabetes mellitus (Cuschieri et al. 2019). T2DM is more typically diagnosed at younger age and body mass index in men, while the most significant risk factor, obesity, is more common in women (Kautzky-Willer et al. 2016). An imbalance between the production of free radicals and the body's natural antioxidant capacity has been linked to oxidative stress disorders, including diabetes (Zatalia and Sanusi, 2013). Obesity is also linked to an increased incidence of Type 2 Diabetes Mellitus and insulin resistance. (T2DM) (Al-Sulaiti et al. 2019).

**2.4 Diagnosis of Diabetes Mellitus**

According to ADA, Diabetes Mellitus can be diagnosed using plasma glucose criteria, includes fasting blood glucose of greater than or equivalent to 126 mg/dl, or plasma glucose more than 200 mg/dl after 2h oral glucose tolerance test or HbA1c greater than or equivalent to 6.5% . A random plasma glucose level of ≥200 mg/dL is diagnostic for people with typical symptoms. (ADA, 2022). Diabetes is diagnosed when one of the criteria said above is met. Patients with typical symptoms require a random plasma glucose level of ≥200 mg/dL for diagnosis. The 2-hour PG produces the highest incidence and lowest A1c levels. A1c is the most convenient and practical test, as it requires no preparation, is analytically superior, and has the lowest intraindividual variability. It costs more than the FPG but equals or less than the OGTT. The 2-hour PG is the most onerous for the patient and has the most intraindividual variance (Genuth et al. 2021). Table 2.2 shows diagnostic criteria for Diabetes Mellitus according to ADA and WHO.

| Criteria | ADA | WHO |
|---|---|---|
| Fasting Plasma Glucose (FPG) | ≥ 126 mg/dl | ≥ 126 mg/dl |
| 2- Hour Plasma Glucose (OGTT) | ≥ 200 mg/dl | ≥ 200 mg/dl |
| Random Plasma Glucose | ≥ 200 mg/dl | ≥ 200 mg/dl |
| HbA1c | Not routinely recommended for diagnosis | ≥ 6.5% |
| Other Requirements | Diagnosis should be confirmed by repeating the test, especially if the patient is asymptomatic | Diagnosis should be confirmed by repeating the test, especially if the patient is asymptomatic |
| Prediabetes - FPG | 110 - 125 mg/dl | 100 - 125 mg/dl |
| Prediabetes - 2-hour Plasma Glucose | 140 - 199 mg/dl | 140 - 199 mg/dl |
| Prediabetes - HbA1c | Not defined | 5.7 - 6.4% |

**Table 2.2 Diagnostic criteria for Diabetes Mellitus according to ADA and WHO**

*FPG- Blood glucose measurement after at least 8 hours of fasting, *OGTT-Blood glucose measurement 2 hours after consuming a 75 g glucose solution, *Random Plasma Glucose- Blood glucose measurement at any time of day, *HbA1c- Glycated hemoglobin, which reflects average blood glucose levels over the past 2-3 months, *Prediabetes- A state in which there is an increase in blood glucose levels over normal but not high enough to be considered diabetes.

### 2.5 Symptoms, Risk factors and Complications of Diabetes

Diabetes symptoms include hyperglycemia, polyuria (frequent urination), polydipsia (excessive thirst), and polyphagia (excessive hunger). Warning signs of diabetes are Dry mouth, Itching, Burning, Pain, numbness on feet, Decreased visions, unexplained weight loss, exhaustion, restlessness, and physical pain, repeated infections especially in the- Genital areas, Urinary tract, skin and delayed healing (Ramachandran, 2014). The main causes of hyperglycemia-induced diabetes are deficiencies in insulin action and impairments in insulin secretion. Additional risk factors for the development of diabetes include a hereditary component, a lifestyle component

(smoking, alcohol intake, and lack of physical activity), gut flora dysbiosis, and a vitamin D and K scarcity (Zimmet et al. 2016; Wu et al. 2014). Many complications can arise from diabetes. The two most common acute consequences are non-ketotic hyperosmolar coma and diabetic ketoacidosis (DKA). Heart disease, stroke, renal failure, ulcers on the feet, and eye impairment are examples of serious long-term consequences. A major function of insulin as an anabolic hormone is the cause of abnormalities in the metabolism of proteins, lipids, and carbohydrates (Balaji et al. 2019).

### 2.6 Obesity and its association with Diabetes Mellitus

Genetics, biology, healthcare availability, mental health, socioeconomic status, personal lifestyle, and environmental factors all play a role, Obesity is now generally recognised as a chronic, relapsing, and multifactorial disease dilatorily jeopardising almost every organ system with its concurrent metabolic disorders or other related comorbidities such as T2DM, cardio- and cerebrovascular diseases, and cancers, impacting both physical and mental health in a variety of ways that cannot be easily solved via weight loss. Obesity is typically defined as having a high BMI. This parameter is more applicable to the population level than defining obesity as a disease, which requires a more comprehensive and systemic assessment (World obesity atlas, 2022). Obesity is increasing the prevalence of type 2 diabetes (T2DM), a chronic disease that causes hyperglycemia due to insufficient insulin production or ineffective insulin use. It is one of the fastest-growing global health emergencies of the century (Malone and Hansen, 2019). Obesity is a contributing factor to the rise in diabetes, which affects nearly 10.5% of the global population and is especially prevalent among youths (IDF, 2022; NCD-RisC,2016; Mayer-Davis, 2017).

Obesity leads to excessive weight gain. Weight gain can lead to metabolic disorders, including T2DM, which is closely linked to obesity. Obesity plays a significant role in the development and progression of metabolism-related T2DM, which is characterised by hyperglycemia due to decreased insulin sensitivity due to reduced functional b-cell mass. Other factors that contribute to this condition include genetic vulnerability, impaired insulin signalling, deteriorated b-cell function, and dysregulated microbiome-gut-brain axis (Malone and Hansen, 2019). T2DM can occur before obesity in individuals with insulin resistance, leading to increased hepatic glucose production and elevated insulin levels, ultimately causing obesity.

T2DM is a slowly progressing metabolic disease linked to obesity. Obesity management can improve or even reverse type 2 diabetes in many patients. Some antidiabetic treatments, such as oral medications, can help reduce body weight, as nearly two-thirds of T2DM patients struggle with obesity. Treatment options for obesity and T2DM include lifestyle interventions (e.g., dietary changes, physical activity, and behavioural therapies), medications, medical devices, and bariatric surgery (Chatterjee et al. 2017).

### 2.7 Aging and its association with Type 2 Diabetes Mellitus

Aging is characterized as a slowdown of physiological activity and a progressive loss of a person's functional ability. It is characterized as a reduced ability to adjust to changes in the environment and internal pressures (Wei et al. 2020). Diabetes, osteoporosis, atherosclerosis, and hypertension are among the senile diseases that emerge with age. Genetic changes, nutritional deficits, changes in mitochondrial function, activation of genes linked to cancer, and other factors are aspects of aging (Li et al. 2019). The prevalence of T2DM and impaired fasting glycemia (IFG) is on the rise due to aging. Furthermore, there is a tendency for the degree of

glucose intolerance to rise with age. The pathophysiology of impaired glucose intolerance in the elderly is influenced by a few specific factors. The main cause is that aging causes changes, lowers insulin sensitivity, and inadequately restores beta-cell activity, all of which contribute to an increase in insulin resistance. Enhanced susceptibility to apoptosis and decreased beta-cell proliferative potential are two conditions that are specifically linked to aging (Halim and Halim, 2019).

According to a study by Szoke et al. 2008, the first and second phases of insulin secretion ordinarily decrease with age at a rate of roughly 0.7% annually; however, in individuals with impaired glucose tolerance, this loss in β cell performance is accelerated by almost two times. However, insulin sensitivity is unaffected by aging in and of itself, regardless of changes in body composition. Insulin resistance may be caused by the loss of lean body mass and the rise in body fat that comes with aging, especially visceral adipocytes or "central obesity." Subclinical inflammation, decreased neuronal stimulation, oxidative stress, and an insulin-resistant state are all factors in the pathophysiology of sarcopenia, or the aging-related loss of muscle mass. Both type 2 diabetes and glucose intolerance are influenced by those conditions (Khamseh et al. 2011). Diabetes mellitus in the elderly (60–65 years of age) is progressively becoming a common and concerning public health issue in both developed and developing nations. According to authors, one of the two elderly people has diabetes or is at risk for developing the disease. Conversely, some authors suggested that eight out of ten individuals have dysglycemia (Chentli et al. 2015).

## 2.8 Insulin Resistance and Its association with Type 2 Diabetes Mellitus

Insulin is an anabolic hormone that can regulate ionic transport, facilitate fluid homeostasis, facilitate the storage of TG in adipose tissue, encourage lipid droplet esterification and storage of fatty acids, and inhibit lipolysis. Insulin is secreted by pancreatic β-cells normally following a meal or the release of hormones such as glucagon and catecholamines (Kumar, 2020). Insulin resistance primarily manifests as symptoms in the liver, muscles, and adipose tissue. Stress, obesity, sedentary lifestyles, pregnancy, poor diet and hunger, and lack of sleep are all factors that can contribute to insulin resistance (Kautzky-Willer et al. 2016). Insulin resistance is characterized by decreased sensitivity and responsiveness to insulin-mediated glucose elimination as well as suppression of the liver's ability to produce glucose. The development of type 2 diabetes (T2DM), which is frequently linked to glucose intolerance, hypertension, visceral obesity, endothelial dysfunction, dyslipidemia, and high levels of inflammatory markers, is primarily caused by insulin resistance (Gutch et al. 2015). Insulin resistance results in hyperinsulinemia due to the overproduction of insulin by pancreatic β-cells. Moreover, hyperglycemia and Type 2 Diabetes are caused by β-cell degeneration in insulin resistance (Gutiérrez-Rodelo et al. 2017).

## 2.9 Uncoupling Proteins

Mitochondrial uncoupling proteins (UCP) are members of a vast family of mitochondrial solute carriers (SLC25s) found in the inner mitochondrial membrane and responsible for transporting protons from the intermembrane gap to the matrix (Kumar et al. 2022). UCPs separate oxidative phosphorylation from ATP synthesis by dissipating energy as heat, also known as the mitochondrial proton leak. UCPs help anions move from the inner to the outer mitochondrial

membrane and protons move from the outer to the inner mitochondrial membrane and decrease the mitochondrial electrochemical potential and plays a crucial role in energy expenditure, activating substrate oxidation, maintaining respiration and prevention of reactive oxygen species (ROS) (Zhao et al. 2019; Vimaleswaran et al. 2011). The figure shows that UCPs consist of six transmembrane domains connected by hydrophilic regions. Each third domain consists of two polar domains and two α-helices.

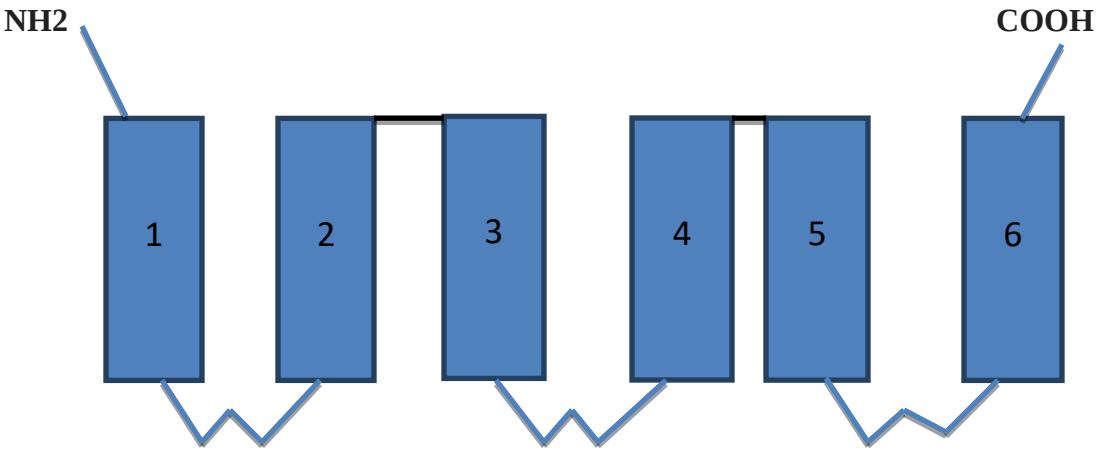



**Figure 2.1: Structure of Uncoupling proteins**

## 2.10 Types of Uncoupling Proteins

The UCP family has been characterized in numerous animals including mammals as consisting of five homologous proteins, UCP1-UCP5. The five mammalian UCP members of the wider SLC25 gene family are numbered in the order in which they were discovered rather than their close relationship (Barnstable et al. 2022). Figure depicts different forms of mitochondrial UCPs and their physiological functions.

### *2.10.1 UCP1*

Brown adipose tissues (BAT) express UCP1 and it is involved in thermogenesis (Li et al. 2008; Gaudry et al. 2018). The uncoupling protein 1 (UCP1), which is found in the mitochondria of brown adipose tissue, lowers the proton concentration gradient created during oxidative phosphorylation, reducing the production of adenosine triphosphate (ATP), and releasing the energy produced as heat radiation(Dong at el. 2020). Adaptive thermogenesis mediated by uncoupling protein 1 (UCP1) protects mammals from hypothermia and metabolic dysregulation (Xue et al. 2022). By increasing glutathione levels, thermogenesis, and energy expenditure, UCP1 can indirectly reduce ROS formation (Čater et al.2022).

### *2.10.2 UCP2*

UCP2 is ubiquitous, expressed in various tissues, including the liver, brain, pancreas, adipose tissue, immunological cells, spleen, kidney, and the central nervous system (Donadelli et al. 2014). UCP2 appears to play a key function in regulating reactive oxygen species generation, reducing inflammation, and inhibiting cell death. UCP2 plays a significant role in the pathophysiology of type 2 diabetes by blocking insulin production in islet beta cells (Mattiasson and Sullivan, 2006).

### *2.10.3 UCP3*

Uncoupling protein 3 (UCP3), a member of the mitochondrial carrier protein is found in brown adipose tissue (BAT), the heart, and muscles (Kreiter et al. 2023). UCP3 is also involved in regulation of free fatty acid metabolism and decrease the reactive oxygen species production by mitochondria and it is also hypothesized to play a defensive role against cardiac ischemia (Souza et al. 2011; Monteiro et al. 2021)

### *2.10.4 UCP4 and UCP5*

UCP4 and UCP5 are expressed mainly in nervous system and perform important functions in energy homeostasis and neuroprotection (Li et al. 2008).

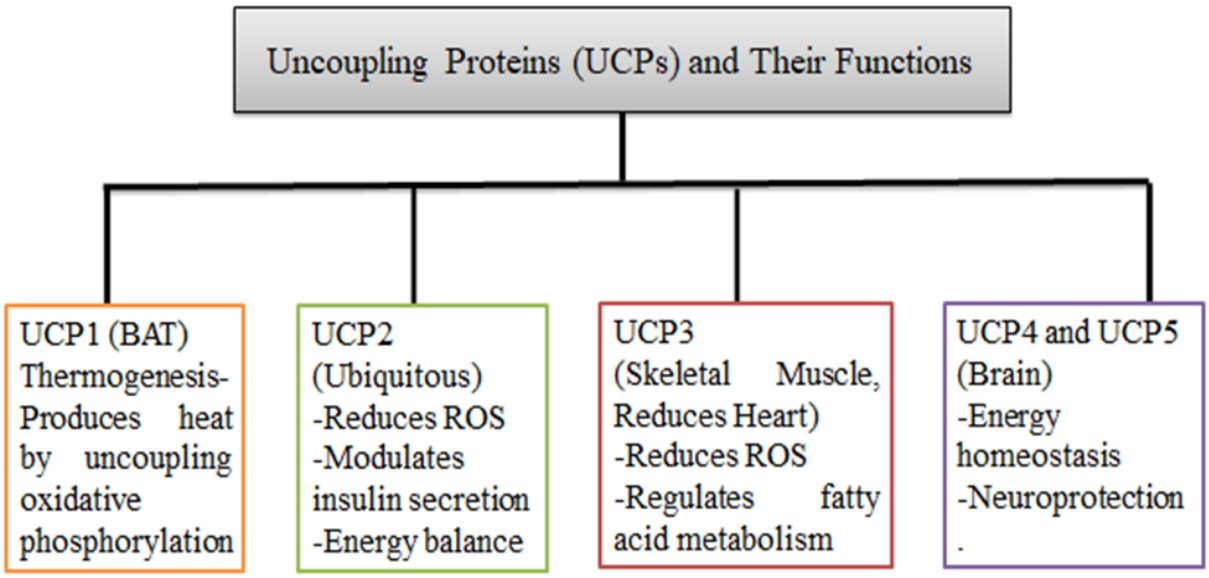

Uncoupling Proteins (UCPs) and Their Functions
UCP1 (BAT) Thermogenesis- Produces heat by uncoupling oxidative phosphorylation
UCP2 (Ubiquitous) -Reduces ROS -Modulates insulin secretion -Energy balance
UCP3 (Skeletal Muscle, Reduces Heart) -Reduces ROS -Regulates fatty acid metabolism
UCP4 and UCP5 (Brain) -Energy homeostasis -Neuroprotection .

**Figure 2.2: Mitochondrial UCPs and their functions**

### 2.11 UCP2 gene relation in pathogenesis of Type 2 Diabetes Mellitus

The UCP2 gene contains eight exons and seven introns and spans a 6.3 kb stretch on chromosome 11 (region 11q13). Numerous organs and cell types, such as skeletal muscle, the brain, the gastrointestinal tract, the pancreatic islets, the liver, the lungs, the spleen, the bone marrow, macrophages, and brown and white adipose tissues, all express UCP2 (Souza et al. 2011). Due to its widespread distribution and strong expression in pancreatic β-cells in diabetes mellitus, UCP2 is the most investigated UCP in relation to its function in the disease. In DM2, there was a strong link between β-cell failure and mitochondrial dysfunction. ROSs trigger UCP2 in β-cells, causing a proton to leak across the inner membrane of the mitochondria. This lowers

β-cell ATP synthesis and content, an important factor in controlling glucose-stimulated insulin secretion (GSIS) (Ma et al. 2012). It is widely believed that a combination of environmental variables and genetic vulnerability have a role in the pathophysiology of type 2 diabetes mellitus (T2DM)(Mattiasson et al. 2006). Hyperglycemia and hyperlipidemia have been shown to promote UCP2 expression, and β-cell function has been reported to be improved and β cell dysfunction to be prevented when UCP2 is genetically deficient (Brand et al. 2010; Kukat et al. 2014). In addition to hyperglycemia and hyperlipidemia, superoxide, a byproduct of reactive oxygen species generated during oxidative phosphorylation, raises UCP2's proton leak activity (Cadenas, 2018). Hence, an increase in UCP2 activity brought on by an increase in lipids, glucose, or ROS levels causes an increase in the stimulation of proton leakage across the mitochondrial membrane, which in turn causes a decrease in ATP synthesis. The reduced synthesis of ATP thus inhibits the release of insulin by β-cells, ultimately resulting in the onset of type 2 diabetes.

### 2.12 UCP2 gene polymorphism in relation with T2DM

The identification of UCP2 in adipose tissue and pancreatic β-cells implies that UCP2 could potentially have a significant impact on the etiology of obesity and Type 2 Diabetes (Jiffri, 2012). The UCP2 promoter region has a single nucleotide polymorphism that is linked to diabetes, hypertension, obesity, and cardiovascular diseases (CVD). It has been shown that a polymorphism in the UCP2 promoter area increases UCP2 expression, which results in decreased insulin release and type 2 diabetes's early development. The G-866A polymorphism of UCP2 has also been connected to increased levels of oxidative stress indicators and differential UCP2 expression (Dalgaard, 2011). Moreover, it has been demonstrated that elevated UCP2 expression reduces insulin production, resulting in early-onset type 2 diabetes. Variations in

UCP2 expression and elevated levels of oxidative stress indicators are linked to the UCP2 promoter -866G/A polymorphism (rs659366). When compared to the G allele, the UCP2 866 A allele is associated with higher levels of UCP2 mRNA expression. Furthermore, the homozygous AA genotype of UCP2 866 is linked to the onset of insulin resistance and the risk of Type 2 Diabetes (Gable et al. 2006; Oguzkan-Balci et al. 2013). Individuals carrying the AA genotype or heterozygous A allele of 866G/A was substantially associated with decreased insulin levels in American, Austrian, Italian and Indian population (Hsu et al. 2008; Krempler et al. 2002; Sesti et al. 2003; Kaul et al. 2015). The A allele of 866G/A is linked to increased promoter activity in beta cells (Krempler et al. 2002), which in turn causes a decrease in ATP synthesis and an increase in UCP2 expression. These outcomes reduce insulin secretion and elevate blood sugar levels. However, in Pima Indian or Japanese populations, there was no correlation discovered between the UCP2 866G/A polymorphism and the incidence of T2DM (Sasahara et al. 2004; Kovacs et al. 2005). The involvement of UCP2 polymorphism in the development of type 2 diabetes warrants more investigation, as it is linked to metabolic syndrome and is a potential candidate gene. This study examines the genetic association between the UCP2 G-866A (rs659366) polymorphism and type 2 diabetes in Delhi NCR population.

# MATERIAL AND METHODS

## RESEARCH SETTING:

Department of Biochemistry, Central Research Facility & Department of General Medicine

Santosh Medical College and Hospital, Ghaziabad -201009

## ETHICAL CONSIDERATIONS:

The necessary approval to conduct the study was obtained from the Santosh Medical College and Hospital, ethical committee, Ghaziabad. Patients were given an explanation regarding the intention of the study and informed written consent was obtained, confidentiality about their results was assured. Their participation was optional.

## PERIOD OF STUDY:

12 Months

## STUDY DESIGN:

Cross sectional study

## SAMPLE SIZE:

This study was a preliminary study. We have taken a total of 60 samples (30 Type 2 Diabetic patients sample and 30 healthy controls)

## INCLUSION CRITERIA:

1. Type 2 Diabetes Mellitus Patients (newly diagnosed with T2DM)
2. Patients who have given written consent for enrolment in the study

**EXCLUSION CRITERIA:**

1. Other types of diabetes (e.g., type 1 diabetes, gestational diabetes)
2. Chronic liver disease, Chronic kidney disease
3. Cardiovascular disease
4. History of cancer
5. Pregnancy or lactation

**DATA COLLECTION:**

A standard questionnaire was framed which included the questions regarding personal data i.e.name of the patient, sex, residence, age, occupation, etc. Before taking blood samples proper consent was taken from diabetic as well as non-diabetic subjects. History was taken and the record was maintained.

**SAMPLE COLLECTION:**

Fasting venous blood samples were collected from 30 T2DM subjects and 30 non diabetic subjects. The blood samples were separated into following vials- sodium fluoride vials for fasting glucose estimation, red topped serum tubes for lipid profile and EDTA vials for DNA extraction. The blood samples in EDTA vials were stored at -20ºC till DNA was extracted.

**BIOCHEMICAL PARAMETERS**

From the collected blood samples serum and plasma were separated by low speed centrifugation for biochemical analysis. Fating blood glucose, HbA1C and serum lipid profile (Total Cholesterol- TC (Cat no: E-BC-K109-S), Triglycerides-TGL (Cat no: E-BC- K238), High Density Lipoprotein-HDL (Cat no: E-BC- K222-S) from (Elabsciences), Low Density

Lipoprotein-LDL and Very Low Density Lipoprotein-VLDL) were measured by using enzymatic method/kit as per manufacturer's protocol.

## DNA ISOLATION FROM HUMAN BLOOD

**Consumables:** Sterile Syringe, fresh blood samples, Vaccutainers (10 ml), micro-pipettes, micro-tips, cotton, tissue paper, disposable gloves, eppendorf (1.5 ml).

### Collection of Samples

2 ml of venous blood samples were collected from all the subjects stored in EDTA were used for DNA isolation.

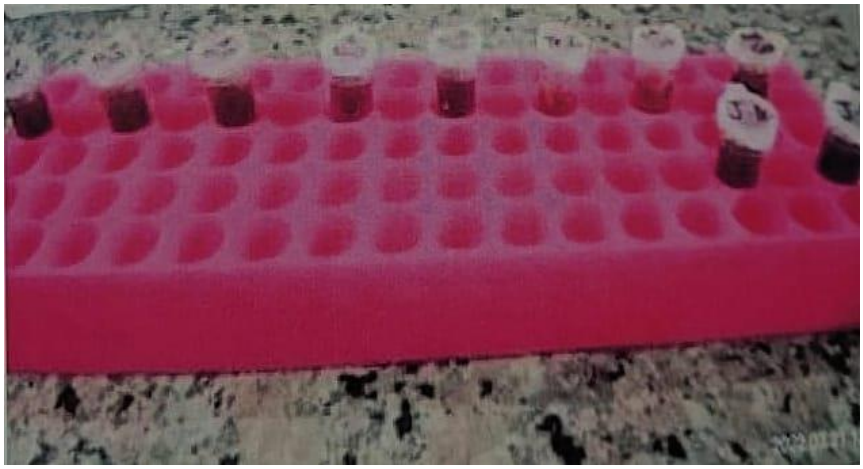


**Fig. 3.1. Blood samples**

**Method of DNA Isolation from human blood**

**Chemicals Required:**

QIAamp DNA Blood mini kit (Qiagen, USA, Cat No. 51104) and Ethanol. Genomic DNA were isolated by using QIAamp DNA Blood mini kit (Olagen, USA, Cat No. 51104) Following protocol was used as per manufacturer's instruction.

**Purification of DNA from Whole Blood**

All centrifugation steps was carried out at room temperature and all the samples, Buffer AE or distilled water was brought to room temperature (15-25°C).

1. 20 µl QIAGEN Protease (or proteinase K) was pipetted into the bottom of a 1.5 ml microcentrifuge tube.
2. 200 µl sample was added to the microcentrifuge tube. Use up to 200 µl whole blood in 200 µl PBS.
3. 200 µl Buffer AL was added to the sample. Mixing was done by pulse-vortexing for 15 s. The sample and Buffer AL was mixed thoroughly to yield a homogeneous solution for efficient lysis
4. The sample and Buffer AL was Incubated at 56°C for 10 min.
5. To remove drops from the inside of the lid centrifugation was done the 1.5ml microcentrifuge tube
6. 200 µl ethanol (96-100%) was added to the sample, and mix again by pulse-vortexing for 15 s. After mixing, we again did centrifuge 1.5 ml microcentrifuge tube to remove drops from the inside of the lid.

7. Mixture was applied carefully to the QlAamp Mini spin column (in a 2 ml collection tube) without wetting the rim. The cap was closed, and centrifuge at 6000 x g (8000 rpm) for 1 min. We put the QIAamp Mini spin column in a clean 2 ml collection tube, and the filtrate was discarded. Each spin column was closed carefully to avoid aerosol formation during centrifugation.
8. The QlAamp Mini spin column was opened carefully and add 500 ul Buffer AW1 without wetting the rim. The cap was closed and centrifuge at 6000 x g (8000 rpm) for 1 min. We place the OlAamp Mini spin column in a clean 2 ml collection tube, and discard the filtrate.
9. We carefully open the QIAamp Mini spin column and 500 ul Buffer AW2 was added. The cap was closed and centrifuge at full speed (20, 000 x g; 14, 000 rpm) for 3 min.
10. It was recommended that the QIAamp Mini spin column was placed in a new 2 ml collection tube and the old collection tube with the filtrate was discarded. Centrifugation was done at full speed for 1 min.
11. Now the QIAamp Mini spin column was placed in a clean 1.5 ml microcentrifuge tube, and the collection tube containing the filtrate was discarded. The QIAamp Mini spin column was opened carefully and 200 µl Buffer AE or distilled water was added. Incubation done at room temperature (15-25°C) for 1 min, and then centrifuge at 6000 x g (8000 rpm) for 1 min.
12. Incubation was done at room temperature for the QIAamp Mini spin column loaded with Buffer AE or water for 5 min before centrifugation to increases DNA yield.
13. A second elution step with a further 200 µl Buffer AE had increased the yields by up to 15%.

14. DNA was eluted in Buffer AE and stored at -30 to -15°C for long-term storage.

## Quantitative and Qualitative analysis of extracted genomic DNA:

To check the quality of DNA, isolated DNA was further studied on 1.0 % agarose gel and UV spectrophotometer.

### a. Agarose Gel Electrophoresis

**Materials:**

a. Agarose

b. TAE buffer (1X) or TBE buffer

c. EtBr [Ethidium Bromide]

d. Loading dye/Tracking dye (6X)- [Bromophenol Blue or Xylene Cynol]

**Instruments:**

Balance, Micro-pipette, Microwave, Electrophoresis Unit (Biorad, USA), Trans illuminator (Biorad, USA) DNA is a negatively charged molecule because of its phosphate group and under electric field it moves towards anode through a matrix of agarose.

**Procedure:**

- 1 gm agarose powder is weighed and added to 100 ml of 1X TAE buffer, then heated in microwave oven until completely melted.
- EtBr [final concentration 0.5µg/ml] is added to the melted agarose when temperature of gel is bearable on hands.
- Melted agarose was poured on pre-placed gel tray.

- Once the gel was solidified, the comb is removed, using care not rip the bottom of the well.
- 2µl of each DNA sample was added to l µl tracking dye and gently mixed with micropipette.
- The lid was closed and power leads were placed on the apparatus. 60V current was applied on the buffer tank.

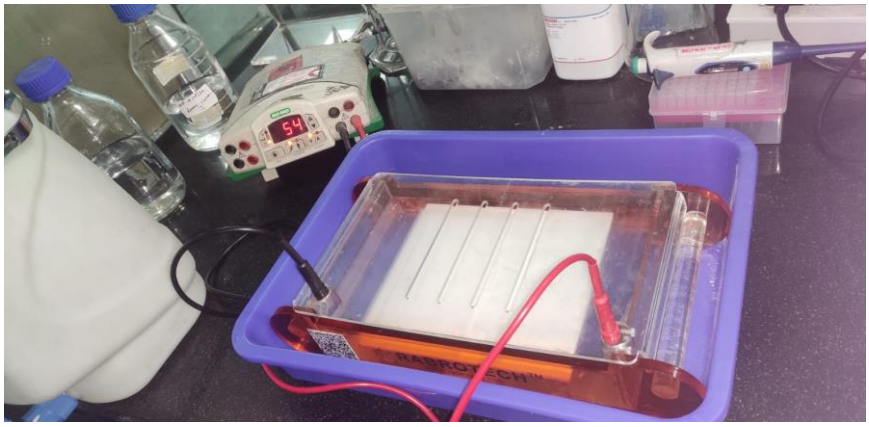


**Fig. 3.2: Horizontal Gel Electrophoresis for DNA samples**

- The distance DNA had migrated in the gel can be judged by visually monitoring the migration of tracking dye.
- Bromophenol blue or Xylene cynol dyes migrate through Agarose gels towards anode.
- When adequate migration has occurred the power supply is switched off and the gel is carefully taken out from the buffer tank place in UV transilluminator.
- As the flourascent dye EtBr interecalates between bases of DNA, the bands of respective DNA fluoresces making a typical pattern of gel.

- Good Quality of DNA will give you an intact band on agarose while sheared DNA will show you a dispersed band.

**Primers used for polymorphic study:**

| S.no. | Gene | Primer Sequences |
|---|---|---|
| a. | UCP2 (F1) | 5'- GTCCTGTGGGGGTAACTGAC – 3' |
| b. | UCP2 (F2) | 5'- GTCCTGTGGGGGTAACTGAT– 3' |
| c. | UCP2 (E) | 5'- GAGACCCAGCGTGACCTC- 3' |
| d. | UCP2 (R) | 5'- CACGCTGCTTCTGCCAGGAC-3' |

**Table 3.1: Primers**

**Analysis of rs659366 (UCP2 -866G/A) Polymorphism**

ARMS-PCR technique was used to study the SNP changes in the UCP2 gene. In this technique reaction was formed in a tube. The ARMS-PCR technique was used to determine the presence of G>A point mutation with primer pairs UCP2 (F1)- 5'- GTCCTGTGGGGGTAACTGAC – 3', UCP2 (F2)- 5'- GTCCTGTGGGGGTAACTGAT– 3', UCP2 (E)- 5'- GAGACCCAGCGTGACCTC- 3' and UCP2 (R)- 5'- CACGCTGCTTCTGCCAGGAC-3' A proliferation fragment was seen in all samples as an indicator of the accuracy of PCR. The expected results of ARMS-PCR in this study included the normal genotype GG, as well as the GA and AA mutant genotypes. After PCR, the products were separated on an agarose gel by electrophoresis and then bands were observed using ultraviolet visualization.

**Amplification Refractory Mutation System Polymerase Chain Reaction (ARMS- PCR)**

The ARMS-PCR (Amplification Refractory Mutation System-PCR) technique is used to detect specific alleles or mutations. This method uses allele-specific primers to amplify only the target allele, differentiating between the wild-type and mutant alleles. The ARMS-PCR technique was employed to determine the presence of the G and A alleles at the -866 position in the UCP2 gene. The PCR reactions were performed in a final volume of 20 µL, which included:

**Fig 3.3: PCR Cycler**

**a. Materials for PCR:**

- 25–50 ng of genomic DNA
- 0.2–0.5 µM of each primer
- 10 µL of 2X PCR master mix (containing Taq DNA polymerase, dNTPs, MgCl2, and buffer)
- Nuclease-free water to the final volume

Each DNA sample was subjected to two separate PCR reactions: one with UCP2 (F1) for the G allele and one with UCP2 (F2) for the A allele

**b. Concentration of Components for 20µl of Reaction:**

| **Component** | **Stock Concentration** | **Final Concentration** | **Volume** |
|---|---|---|---|
| **DNA Template** | **200ng/µl** | **100ng-1µl** | **1**µl |
| Buffer | 10X | 1X | **2**µl |
| dNTPs | 10mM of each | 200µM of each | **0.4**µl |
| Taq Polymerase | 3U/ µl | 1 Unit | **0.33**µl |
| Primers | 100pm/µl | 0.05µM | **2**µl |
| MgCl2 | 20mM | 1.5mM | **-** |
| Nuclease free water (NFW) | ……. | To make Volume upto 20 µl | **14.27**µl |
| | | | **Total 20**µl |

**Table 3.2: Concentration of components for PCR**

**PCR CYCLING CONDITIONS**

The amplification was carried out in a thermo cycler under the following cycling conditions:

- Initial denaturation at 95°C for 5 minutes

35 cycles of:

- Denaturation at 95°C for 30 seconds
- Annealing at 57°C for 30 seconds
- Extension at 72°C for 30 seconds

- Final extension at 72°C for 5 minutes

The PCR products were then held at 4°C until further analysis.

**Gel Electrophoresis**

The amplified products were analyzed using 2% agarose gel electrophoresis. Gels were prepared by dissolving 2 g of agarose in 100 mL of 1X TBE buffer and stained with ethidium bromide (0.5 μg/mL). A 5 μL aliquot of each PCR product was mixed with 1 μL of 6X loading dye and loaded into the wells of the gel alongside a 100 bp DNA ladder as a size reference. Electrophoresis was performed at 100 V for 45 minutes in 1X TBE buffer.

**Visualization and Genotyping**

The gels were visualized under ultraviolet (UV) light. The presence of a specific PCR product indicated the presence of the corresponding allele.

Samples were classified as homozygous G/G, homozygous A/A, or heterozygous G/A based on the pattern of bands observed.

## STATISTICAL ANALYSIS

Data were analyzed by using IBM SPSS 26 software. The results for continuous variables were expressed as mean ± standard deviation and normally distributed variables were analyzed using student's t-test. Chi- square was used to test the association in genotypes frequency between the groups. Logistic regression analysis was used to calculate the odds ratio to test the association of genotypes and T2DM, keeping the wild type as the reference. The association of -866G/A UCP gene polymorphism with other T2DM factors was tested by using multiple logistic regression analysis, assessing the independence from potential cofounders. P value less than 0.05 considered as significant.

## RESULTS

The present preliminary study was performed to evaluate the association of UCP2 gene with development of T2DM in Delhi NCR population. The collection of data was done for 12 months. The study involved 60 individuals including 30 T2DM subjects and 30 control subjects. The diagnostic criterion of the American Diabetic Association (ADA, 2010) was followed to diagnose subjects with T2DM. Further, the T2DM subjects were categorized according to age, gender and BMI and comparison was made to non-diabetic healthy subjects. The demographic features of study population are shown in Table 4.1.

**Table 4.1: Demographic features of the study population**

| **Demographic feature** | **T2DM subjects (n=30)** | **control (n=30)** |
|---|---|---|
| **Males** | 17 | 19 |
| **Females** | 13 | 11 |
| **Age≥50 years** | 19 | 12 |
| **Age<50 years** | 11 | 18 |
| **BMI (kg/m$^2$)** | | |
| BMI 18.5-24.9 (normal) | - | 6 |
| BMI ≥25.0-29.9 (overweight) | 21 | 22 |
| BMI ≥30 (obese) | 9 | 2 |

### Characteristic features (biochemical and clinical parameters) of the study participants

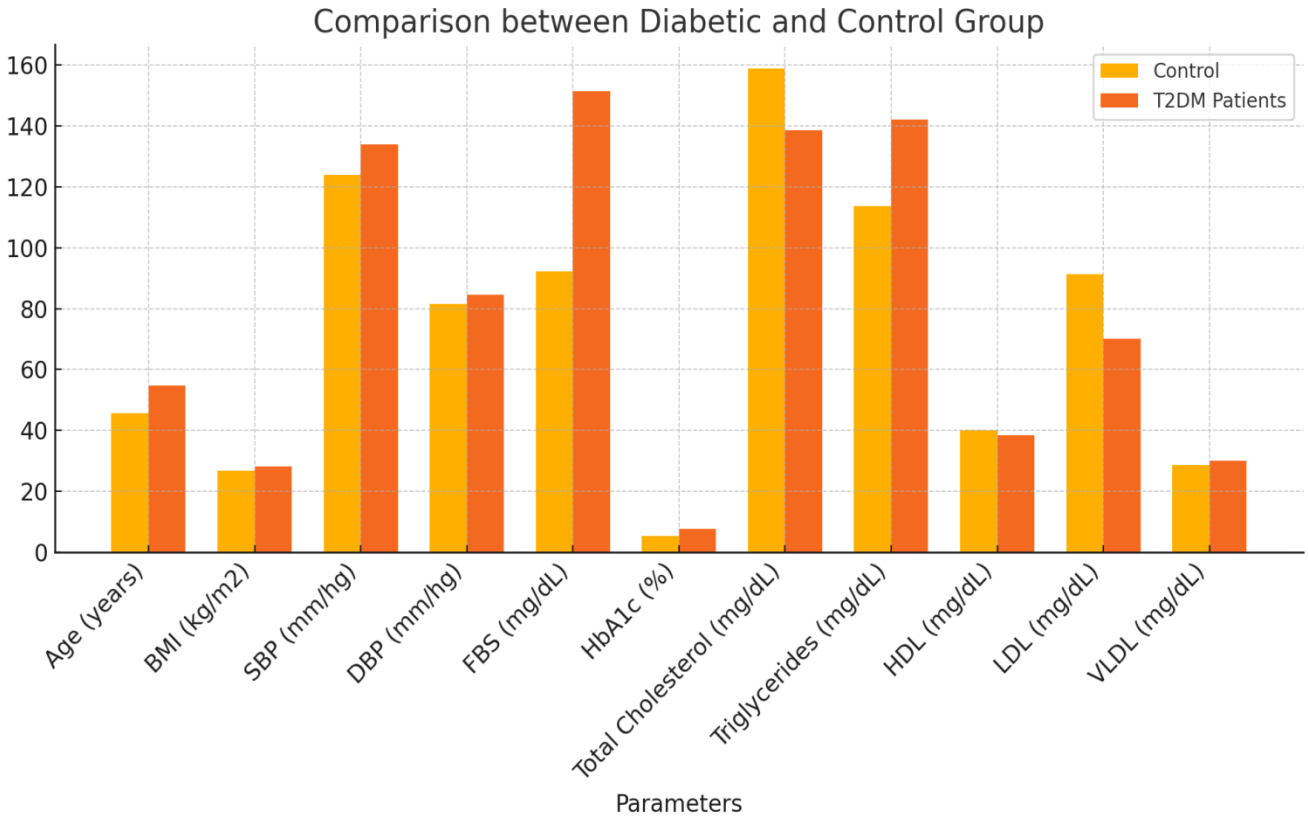
The biochemical and clinical parameters of all the study subjects were measured.The Independent t-test analysis showed that the fasting blood sugar (FBS), glycated hemoglobin (HbA1c), low density lipoprotein(LDL), Triglycerides, body mass index (BMI), systolic blood

pressure (SBP), and diastolic blood pressure (DBP) levels in T2DM subjects were significantly higher than non-diabetic subjects ($p < 0.05$; Table 4.2).

**Table 4.2: Biochemical parameters and clinical features of study participants**

| Parameter | Control | T2DM patients | p- value |
|---|---|---|---|
| **Age (years)** | 45.6±12 | 54.7±13.8 | 0.008 |
| **BMI ($kg/m^2$)** | 26.8±2.4 | 28.2±2.1 | 0.02 |
| **SBP (mm/hg)** | 123.8±8.9 | 134±12.2 | 0.0005 |
| **DBP (mm/hg)** | 81.6±5.1 | 84.6±4.7 | 0.02 |
| **FBS (mg/dL)** | 92.2±8.7 | 151.3±65.1 | <0.0001 |
| **HbA1c (%)** | 5.3±0.25 | 7.6±1.7 | <0.0001 |
| **Total Cholesterol (mg/dL)** | 158.9±38 | 138.6±45.1 | 0.06 |
| **Triglycerides (mg/dL)** | 113.7±49.6 | 142.1±47.7 | 0.02 |
| **HDL (mg/dL)** | 40±4.9 | 38.4±4.9 | 0.21 |
| **LDL (mg/dL)** | 91.4±36.1 | 70.1±40.6 | 0.03 |
| **VLDL (mg/dL)** | 28.7±13.2 | 30±13.8 | 0.71 |

The table shows the biochemical parameters and clinical characteristics of the Type 2 Diabetes Subjects and non-diabetic subjects. The Data are shown as mean ± SD, analyzed by independent t- test, $p<0.05$ was considered as statistically significant. BMI: body mass index; SBP: systolic blood pressure: DBP: diastolic blood pressure; FBS: fasting blood sugar; HDL: high density lipoprotein; LDL: low density lipoprotein; VLDL: very low density lipoprotein.




**Evaluation of genomic DNA obtained from T2DM subjects and normal healthy controls**

The Genomic DNA was extracted from whole blood samples of 30 T2DM subjects and 30 normal healthy controls. The genomic DNA so obtained was checked for integrity by loading 2µl of DNA on 1% agarose gel. DNA in the gel was visualized under UV light.

**Determination of UCP2 -866G/A (rs659366) single nucleotide polymorphism**

ARMS-PCR technique was used to study the SNP changes in the UCP2 gene. In this technique reaction was formed in a tube. The ARMS-PCR technique was used to determine the presence of G>A point mutation with primer pairs.

A proliferation fragment was seen in all samples as an indicator of the accuracy of PCR. The expected results of ARMS-PCR in this study included the normal genotype GG, as well as the GA and AA mutant genotypes. After PCR, the products were separated on an agarose gel by electrophoresis and then bands were observed using ultraviolet visualization (Fig 4.1 to 4.12)

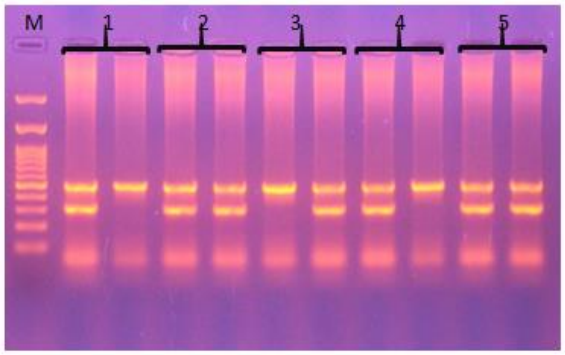



**Fig 4.1**

| T2DM | Genotype |
|---|---|
| 1 | GG |
| 2 | GA |
| 3 | AA |
| 4 | GG |
| 5 | GA |

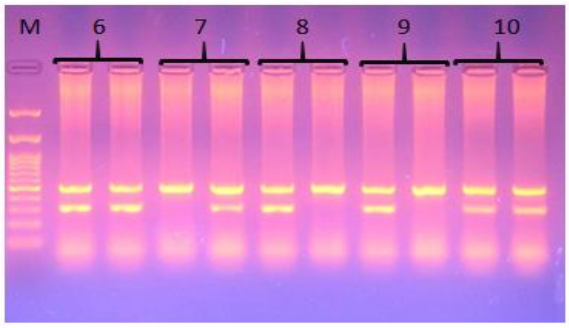



**Fig 4.2**

| T2DM | Genotype |
|---|---|
| 6 | GA |
| 7 | AA |
| 8 | GG |
| 9 | GG |
| 10 | GA |

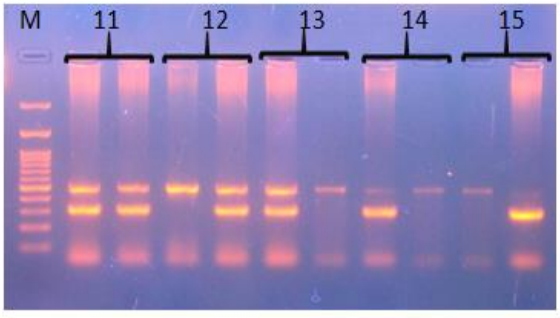



**Fig 4.3**

| T2DM | Genotype |
|---|---|
| 11 | GA |
| 12 | AA |
| 13 | GG |
| 14 | GG |
| 15 | AA |

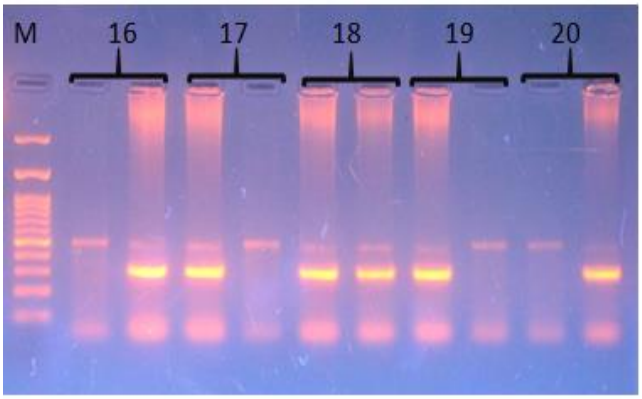



**Fig 4.4**

| T2DM | Genotype |
|---|---|
| 16 | AA |
| 17 | GG |
| 18 | GA |
| 19 | GG |
| 20 | AA |

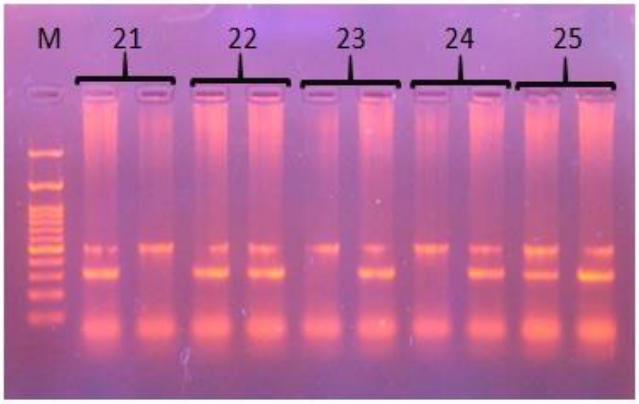



**Fig 4.5**

| T2DM | Genotype |
|---|---|
| 21 | GG |
| 22 | GA |
| 23 | AA |
| 24 | AA |
| 25 | GA |

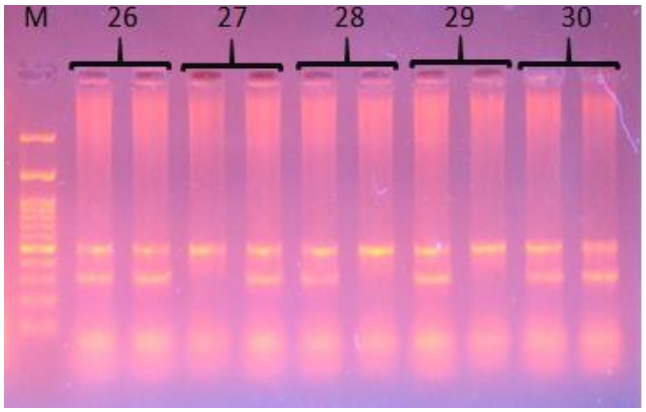



**Fig 4.6**

| T2DM | Genotype |
|---|---|
| 26 | GA |
| 27 | AA |
| 28 | GG |
| 29 | GG |
| 30 | GA |

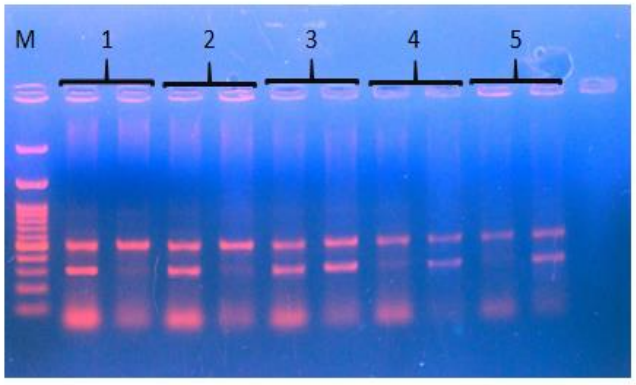



**Fig 4.7**

| Control | Genotype |
|---|---|
| 1 | GG |
| 2 | GG |
| 3 | GA |
| 4 | GA |
| 5 | AA |

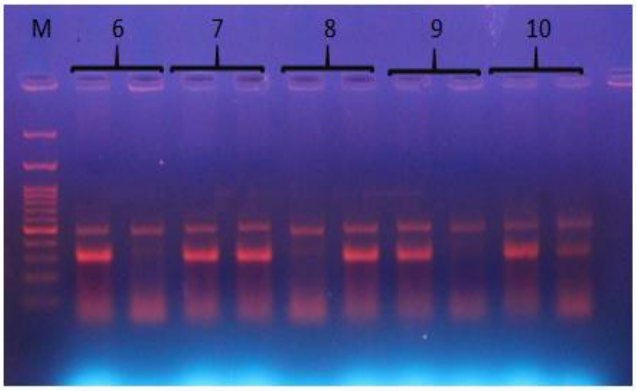



**Fig 4.8**

| Control | Genotype |
|---|---|
| 6 | GG |
| 7 | GA |
| 8 | AA |
| 9 | GG |
| 10 | GA |

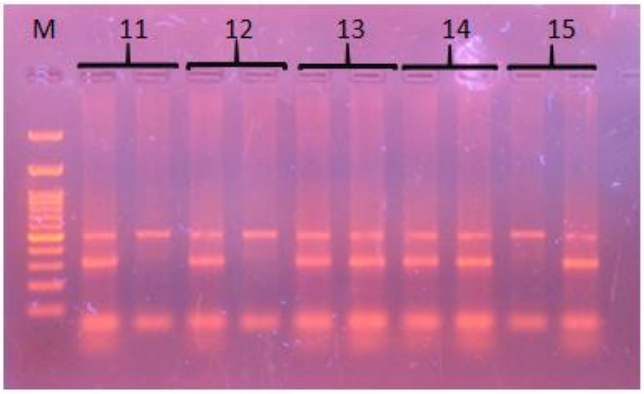



**Fig 4.9**

| Control | Genotype |
|---|---|
| 11 | GG |
| 12 | GG |
| 13 | GA |
| 14 | GA |
| 15 | AA |

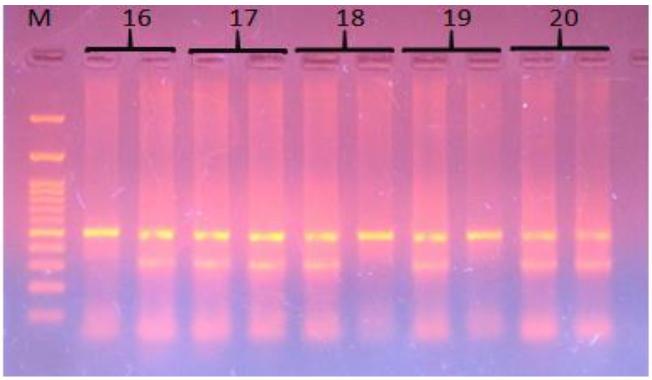



**Fig 4.10**

| Control | Genotype |
|---|---|
| 16 | AA |
| 17 | GA |
| 18 | GG |
| 19 | GG |
| 20 | GA |

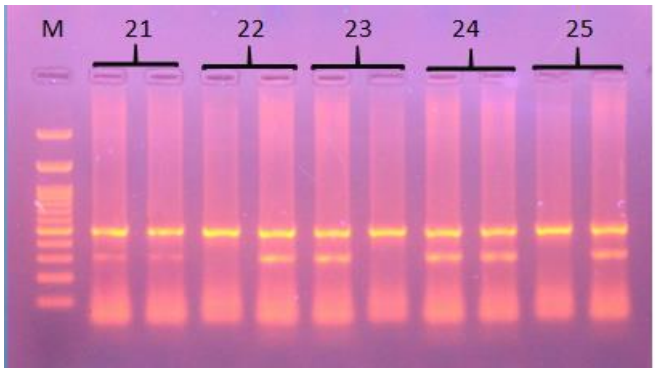



**Fig 4.11**

| Control | Genotype |
|---|---|
| 21 | GA |
| 22 | AA |
| 23 | GG |
| 24 | GA |
| 25 | AA |

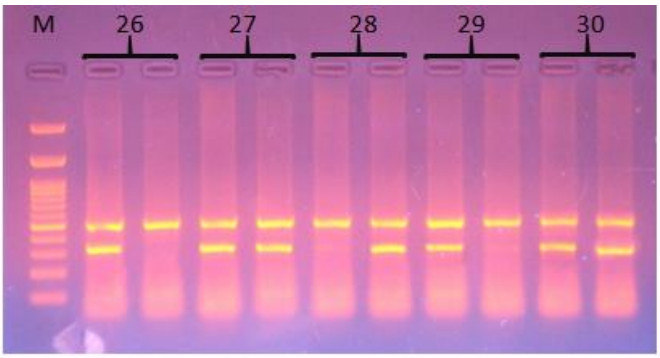



Fig 4.12

| Control | Genotype |
|---|---|
| 26 | GG |
| 27 | GA |
| 28 | AA |
| 29 | GG |
| 30 | GA |

**Genotype and Allele frequencies of T2DM patients vs controls for -866G/A polymorphism in UCP2 gene**

The distribution of genotype between the control group, n = 30, and T2DM patient group, n = 30, was tested. The GG genotype was found in 11 individuals, 36.66%, both in the control and T2DM groups, and it was thus the reference category. The GA genotype was found in 13 individuals, 43.33%, in the control group, and 10 individuals, 33.33%, in the T2DM group. The odds ratio of the GA genotype in T2DM patients compared with the control group was 0.76 (95% CI: 0.23–2.48, p=0.661); thus, no significant statistical difference was observed.

Among controls, 6 (20%) had the AA genotype, while in the T2DM group, 9 subjects possessed this genotype accounting for 30% of cases. In T2DM patients, the estimated OR for the AA genotype was 1.5 [95% CI: 0.39-5.66, p=0.550], which also did not reach any statistical significance between the two groups.

Allele frequencies were also considered. The G allele was carried by 58.33% of the control and 53.33% of the T2DM, which was used as the reference allele. The A allele was found in 41.66% of the control and 46.66% of the T2DM. The odds ratio of the A allele in T2DM subjects was 0.81 (95% CI: 0.39–1.68, p=0.581), which means that there is no significant difference between the groups in allele distribution.

**Table 4.3 Genotype and allele frequency distribution in control and T2DM patients**

| Genotype | Control (n=30)% | T2DM patients (n=30)% | OR (95% CI) | p-value |
|---|---|---|---|---|
| GG[a] | 11 (36.66) | 11 (36.66) | Reference | |
| GA | 13 (43.33) | 10 (33.33) | 0.76 (0.23 to 2.48) | 0.661 |
| AA | 6 (20) | 9 (30) | 1.5 (0.39 to 5.66) | 0.550 |
| **Allele** | | | | |
| G[a] | 35 (58.33) | 32 (53.33) | Reference | |
| A | 25 (41.66) | 28 (46.66) | 0.81 (0.39 to 1.68) | 0.581 |

OR = odds ratio; CI = confidence interval; [a] Reference category;comparison between group was performed with Chi-square test.

**Association of UCP2 -866G/A polymorphism with anthropometric and biochemical parameters in T2DM patients**

The biochemical and clinical profile of study population by UCP2 genotype among T2DM cases for the - 866G/A polymorphisms are shown in Table 4.5. The GG, GA, and AA groups consisted of 11, 10, and 9 participants, respectively. The analysed variables include BMI, SBP, DBP, FBS, HbA1c levels, and lipid profile (total cholesterol, triglycerides, HDL, LDL, and VLDL).

The mean BMI values among the different genotypes were quite close,the mean SBP in the GG genotype was highest at 137.73±13.1 mmHg, followed by the AA genotype at 134.44±12.85 mmHg and the GA genotype at 129.5±10.39 mmHg.DBP was also similar across the groups, The FBS levels were higher in the group with GG genotype, in comparison with the group with GA genotype, HbA1c is a marker for long-term blood sugar control. The values ranged as 8.06±2.39% for GG, 6.82±0.58% for GA, and 7.93±1.34% for AA. Though the values showed variation, the p-value of

0.202 reflects that these variations are not significant.Values of total cholesterol were noted to be on the higher side in AA followed by GA then GG,HDL levels difference among the genotypes were almost similar. LDL level was also higher in AA genotype.

The analysis provides evidence that there is no statistically significant difference in biochemical and clinical characteristics of T2DM patients with respect to UCP2 genotype for the -866G/A polymorphism. All the p-values are >0.05, meaning the genotype does not contribute significantly to the variation in the measured variables within this study population.

**Table 4.4 Biochemical and clinical characteristics of the study population according to UCP2 genotype for -866G/A polymorphism in T2DM patients**

| Variables | GG (11) | GA (10) | AA (9) | p-value |
|---|---|---|---|---|
| **BMI (kg/m$^2$)** | 28.03±2.12 | 28.54±2.33 | 28.22±2.29 | 0.858 |
| **SBP (mm/hg)** | 137.73±13.1 | 129.5±10.39 | 134.44±12.85 | 0.316 |
| **DBP (mm/hg)** | 86.82±5.13 | 83±4.21 | 83.89±4.16 | 0.152 |
| **FBS (mg/dL)** | 173.66±90.8 | 130.74±27.70 | 147.03±54.77 | 0.323 |
| **HbA1c (%)** | 8.06±2.39 | 6.82±0.58 | 7.93±1.34 | 0.202 |
| **Total Cholesterol (mg/dL)** | 132.55±42.09 | 141.3±48.22 | 143±49.7 | 0.861 |
| **Triglycerides (mg/dL)** | 162.22±56.46 | 147.95±44.10 | 143.15±105.17 | 0.822 |
| **HDL (mg/dL)** | 38.61±3.08 | 38.08±2.82 | 38.5±4.03 | 0.969 |
| **LDL (mg/dL)** | 62.2±40.13 | 73.71±43.85 | 75.86±40.77 | 0.727 |
| **VLDL (mg/dL)** | 31.62±11.01 | 29.5±8.95 | 28.63±21.03 | 0.888 |

Values are mean ± SD; Genotypes were compared by one-way ANOVA. BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; FBS: Fasting blood sugar; HDL: high density lipoprotein; LDL: low density lipoprotein; VLDL: very low density lipoprotein

# DISCUSSION

The present study was planned to study the association between the -866G/A polymorphism in the UCP2 gene and T2DM in a sample population drawn from the Delhi NCR region as theoretically UCP2 is known to the regulate the energy metabolism and decrease oxidative stress, which is of prime importance in the pathophysiology of T2DM.

There was no significant difference in genotype distribution and allele frequencies as observed between T2DM patients and healthy controls. Moreover, the odds ratio related to GA and AA genotypes were 0.76 (95% CI: 0.23–2.48, p=0.661) and 1.5 (95% CI: 0.39–5.66, p=0.550), respectively, showing no significant elevation of the risk for T2DM. The A allele was slightly more frequent in T2DM patients, but this difference did not reach statistical significance. Thus, the A allele did not significantly increase susceptibility for T2DM: OR=0.81, 95% CI: 0.39–1.68, p=0.581.

These findings were consistent with the studies that find no significant associations between UCP2 polymorphisms and the risk of T2DM. Nevzat et al. 2017 and Kovacs et al. 2015 in a study done in Turkish population and Pima Indian respectively observed that there was no significant association of UCP2 -866G/A polymorphism and T2DM, though at the hypothesis phase they had assumed otherwise. Also, no relation of UCP2 variants to obesity or diabetes was revealed in the European cohort study by Esterbauer et al. 2001 and meta-analysis study by De Souza et al. 2013 on European ancestory showed that -866G/A UCP2 gene polymorphism is not associated with T2DM. this further points to the possibility that the effects of UCP2 polymorphisms on metabolic disorders could be population-specific or modulated by other genetic and environmental factors.

Besides the genetic analysis, we constructed the biochemical profile of the patients with T2DM according to the different UCP2 genotypes. The major metabolic parameters did not differ significantly among the genotypes regarding BMI, blood pressure, FBS, HbA1c, and lipid profiles. For instance, the p-value regarding BMI within the genotypes was 0.858, indicating that the -866G/A polymorphism has no significant effect on that parameter.

These findings are therefore in agreement with other reports, which equally had no significant influence of the UCP2 -866G/A polymorphism on metabolic traits in patients with T2DM. Gomathi et al. 2019 studied a South Indian population and found no significant association of the variant lipid profiles. Similarly, D'Adamo et al. 2009 did not find any significant impact of UCP2 variants on glycemic control or insulin sensitivity in a study involving Italian adolescents.

Although the main attention of the study was directed at the association of the -866G/A polymorphism of the UCP2 gene with T2DM, a number of biochemical and clinical data were also analyzed, including BMI, blood pressure, FBS, HbA1c, and lipid profiles. In the T2DM patients, the study found that levels of fasting blood sugar, HbA1c, systolic and diastolic blood pressure, and BMI were significantly higher as compared to the nondiabetic controls ($p < 0.05$). This agrees with known clinical characteristics of T2DM and lends further confidence to the association between these parameters and diabetes regardless of UCP2 polymorphism.

However literature search showed no studies in north Indian population of Delhi NCR. The results other ethnic globally and nationally showed significant correlation with gene -866G/A UCP2 polymorphism with Type 2 Diabetes Mellitus. However several reasons may account for the non-significant associations observed in this study. First, the relatively limited sample size

in this study (n = 30) may limit the statistical power necessary to detect subtle genetic effects. Second, since T2DM is a complex disease involving interactions of multiple genes and environmental factors, it is probable that any one single polymorphism, such as the -866G/A of the UCP2 gene, might contribute with only a modest effect, difficult to observe in a small study. Further research should be conducted on larger and more diverse populations in order to confirm these results and explore potential gene-gene and gene-environment interactions.

# CONCLUSION

The findings of the present preliminary study do not support any significant association between the UCP2 -866G/A polymorphism and T2DM development among the Delhi NCR population. The lack of significant association under all the genetic models, therefore, suggests that this may or may not be a major modifier of risk to T2DM in this small population (n=30). Larger sample size and more extensive studies on genetics are suggested to elucidate the exact role of UCP2 and other genetic factors in the pathogenesis of T2DM.

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# PROFORMA

| DEMOGRAPHIC DATA | |
|---|---|
| **Name** | **Mr./Mrs./Ms.:-** |
| **Date of Birth:-** | **Sex: Male/Female** |
| **Weight (in Kg):-** | **Height (in cm):-** |
| **Address:**<br>**Street: _____ City: _____**<br>**Stare: _____ Pin Code: _____** | |
| **Mobile No.:** | **Email:** |
| **Education Status:** | |
| **Occupation:** | |
| **At which age T2DM diagnosed?** | |

## SANTOSH DEEMED TO BE UNIVERSITY (GHAZIABAD)

## INFORMED CONSENT

I……Son/Daughter/Wife of……resident of……hereby declare that I give my full, free and voluntary informed consent in the thesis ASSOCIATION OF THE -866G/A POLYMORPHISM IN THE UCP2 GENE WITH TYPE 2 DIABETES MELLITUS. Investigator ……has informed me to my full satisfaction, in the language I understand, about the purpose, nature of study and various laboratory investigations for the study to be carried out. – I have been informed about the duration of the study and instructions to be followed during the study period.

I give full consent for being enrolled in the above study and I reserve my rights to withdraw from this study, whenever I wish without prejudice of my right to undergo further treatment at Santosh Hospital, Ghaziabad U.P

Patient's/ Patient's relative's signature Doctor’s signature

Or thumb impression

Name: Date:

We have witnessed that the patient signed the above form in the presence of his free will after fully understanding its content.

Signature of Witness

Name: Signature of Investigator

Name – Ayushi Vishnoi

Designation- Student

(Msc Medical Biochemistry, 2021-2024)

Contact No- 9634566011

## संतोष डीम्ड यूनिवर्सिटी (गाज़ियाबाद)

## सहमति पत्र (सुचित)

मैं.....बेटा/बेटी/ पत्नी.....निवासी.....घोषण करता हूं कि शोधकता .....ने मुझे मेरी भाषा में अपने अध्ययन के उद्देश्य के बारे में और अध्ययन के दौरान जाने अपनायी जाने वाली विभित्र प्रयोगशाला जांच के बारे में सूचित कर दिया है। मुझे अध्ययनकी अवधि और उसके दौरान अपनाए जाने वाले निर्देश भी मेरी भाषा में समझा दिए गए हैं। मैं उपरोक्त अध्ययन में बिना किसी दबाव के दाखिला ले रहा हूँ और पूर्ण सहमति दे रहा हूं। सन्तोष अस्पताल, गाजियाबाद में अपने आगे के उपचार पर बिना प्रतिकूल प्रभाव डाले इस अध्ययन से और प्रयोगशाला जांच की प्रकृति से में जब चाहूँ बाहर जाने के अपने अधिकार को सुरक्षित रख रहा हूं।

रोगी के रिश्तेदार के हस्ताक्षर

डॉक्टर के हस्ताक्षर।

नाम

दिनांक:

मेरी उपस्थिति में मरीज ने पूरी तरह से डाकटर के बताए गए अध्ययन और निर्देशों को समझने के बादअपनी स्वतंत्र इच्छा से ऊपर के फार्म पर हस्ताक्षर किए हैं।

हस्ताक्षर

नाम: .....

पदनामः .....

संपर्क नंबर: .....

## DETAILS OF PATIENT INFORMATION SHEET

## HUMAN RESEARCH PROPOSAL

1. Identifying Data

Name of Researcher: Ayushi Vishnoi

Department: Biochemistry

Title of Project: ASSOCIATION OF THE -866G/A POLYMORPHISM IN THE UCP2 GENE WITH TYPE 2 DIABETES MELLITUS

2. Description of Human Subjects Safeguards and Rights:

a) Possible psychological of physical risk of discomfort, involved in research If Yes/No (if no, skip time B)
If yes, answer the following

Why necessary?

1. To have the common disease
2. Specificity and sensitivity of the treatment

b) Make sure the following questions are answered in your research proposal on collection of Data:
1) When will it take place?
2) What length of time needed for complete examination of the patient?
3) What period of the time needed?
4) Which specific place within institution?
5) How much population required including number of subjects?
6) Name of instrument to be used for data collection?
7) Methodology to be used (write details)?

c) Will subjects be given research results if they so request? Yes / No

# LETTER OF ETHICAL CLEARANCE FOR DISSERTATION

# FROM REGISTRAR OFFICE

**SANTOSH**
**Deemed to be University**
**(Established u/s 3 of the UGC Act, 1956)**

F. No. SU/R/2024/1344[6] Dated: 17.05.2024

Subject: Grant of Ethical Clearance for **Dissertation Protocol** Topic **"Association of the 866G/A Polymorphism in the UCP2 Gene",** submitted by **AYUSHI VISHNOI,** student of M.Sc. Medical BIOCHEMISTRY, 2021 Batch.

*****

With reference to the request for grant of Ethical Clearance for the Dissertation Protocol entitled **"Association of the 866G/A Polymorphism in the UCP2 Gene"**, **AYUSHI VISHNOI** is informed that the **Dissertation Protocol** submitted by him/her was considered by the **Screening Committee** of the **Allied Health Sciences** in its meetings held on 07.02.2024 and 29.02.2024. The recommendations of the **Screening Committee** were considered in detail by the **Institutional Ethics Committee** in its meeting held on **15.05.2024** and the same was **approved** by the **Ethics Committee** of the **Santosh Deemed to be University, Ghaziabad, Delhi NCR**.

He /She is informed accordingly for further necessary action.

17/5/24

**[Dr. EZHILRAJAN. K]**
**REGISTRAR**

**AYUSHI VISHNOI**
**M.Sc. Medical BIOCHEMISTRY.**

**Copy to:**

1. The Vice Chancellor
2. The Dean – Academics
3. The Dean, SMC&H
4. The Dean – Research
5. The Director - IQAC
6. HOD/ Course In-charge of the concerned Department.

# STATISTICIAN CERTIFICATE

This is to certify that the statistical analysis component of the thesis titled “ASSOCIATION OF THE -866G/A POLYMORPHISM IN THE UCP2 GENE WITH TYPE 2 DIABETES MELLITUS” done by Ayushi Vishnoi in the Department of Central Research Facility, Santosh Medical College and Hospital, Ghaziabad has been conducted under my direct guidance and supervision.

All the statistical calculations of this study are authentic and correct to the best of my knowledge.

Wish her all the success in her life.

Dr. Amod Kumar

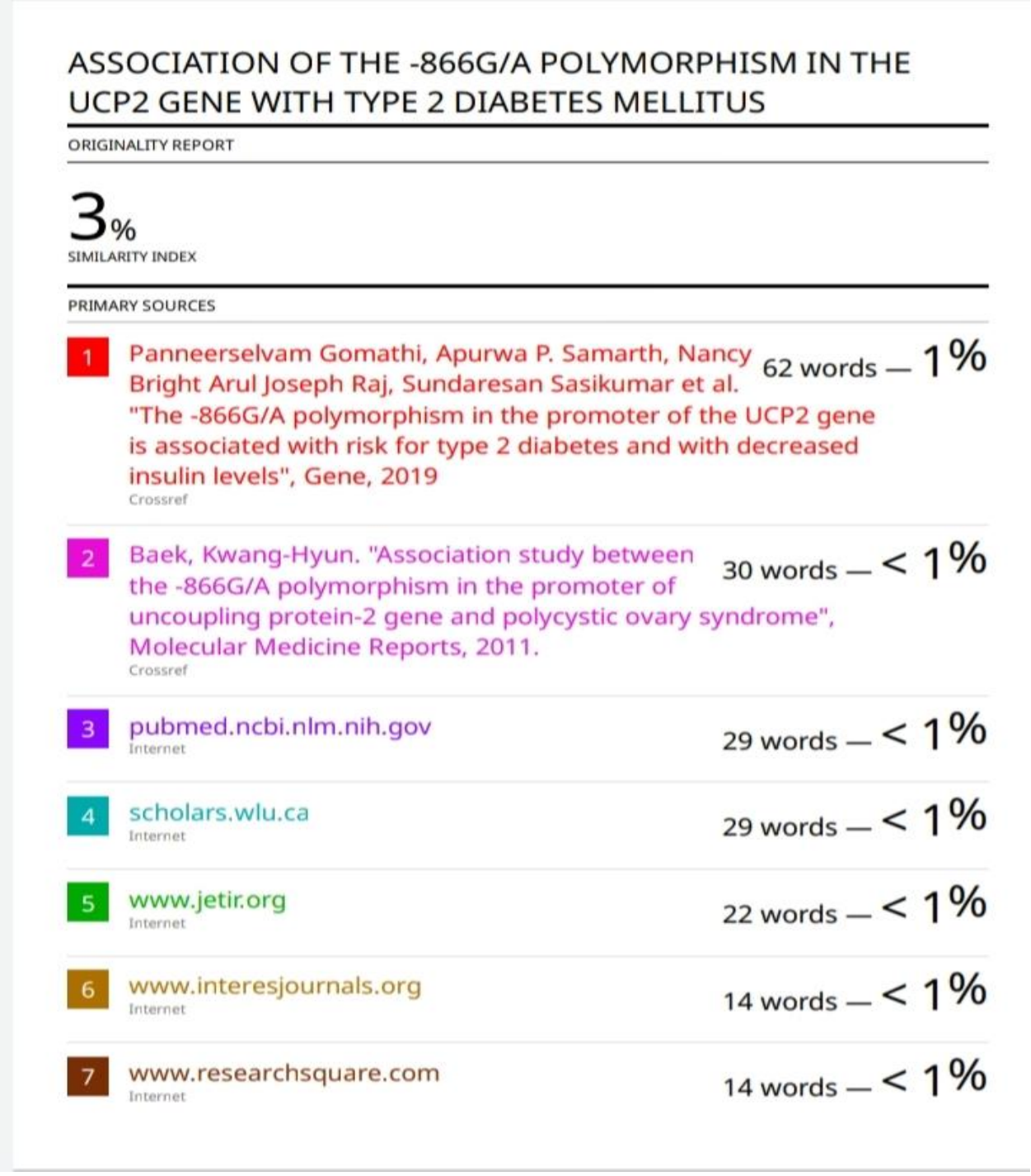
Assistant Professor cum Statistician

Department Of Community Medicine

Santosh Deemed To be University, Ghaziabad.

Email : Amod.ism01@gmail.com

# PLAGIARISM CERTIFICATE



ASSOCIATION OF THE -866G/A POLYMORPHISM IN THE UCP2 GENE WITH TYPE 2 DIABETES MELLITUS

ORIGINALITY REPORT

3%
SIMILARITY INDEX

PRIMARY SOURCES

| | | |
|---|---|---|
| 1 | Panneerselvam Gomathi, Apurwa P. Samarth, Nancy Bright Arul Joseph Raj, Sundaresan Sasikumar et al. "The -866G/A polymorphism in the promoter of the UCP2 gene is associated with risk for type 2 diabetes and with decreased insulin levels", Gene, 2019<br>Crossref | 62 words — 1% |
| 2 | Baek, Kwang-Hyun. "Association study between the -866G/A polymorphism in the promoter of uncoupling protein-2 gene and polycystic ovary syndrome", Molecular Medicine Reports, 2011.<br>Crossref | 30 words — < 1% |
| 3 | pubmed.ncbi.nlm.nih.gov<br>Internet | 29 words — < 1% |
| 4 | scholars.wlu.ca<br>Internet | 29 words — < 1% |
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# MASTER CHART

## Master chart for demographic parameters of T2DM patients

| Sample I.D. | Gender | Age | Height (cm) | Weight (kg) | BMI | SBP | DBP |
|---|---|---|---|---|---|---|---|
| T2DM001 | Male | 48 | 170 | 88 | 30.4 | 120 | 80 |
| T2DM002 | Female | 40 | 160 | 68 | 26.6 | 115 | 75 |
| T2DM003 | Female | 80 | 155 | 72 | 30 | 130 | 80 |
| T2DM004 | Male | 64 | 168 | 90 | 31.9 | 140 | 85 |
| T2DM005 | Female | 53 | 158 | 70 | 28 | 125 | 80 |
| T2DM006 | Male | 77 | 172 | 95 | 32.1 | 130 | 85 |
| T2DM007 | Female | 65 | 162 | 76 | 29 | 145 | 85 |
| T2DM008 | Male | 51 | 175 | 85 | 27.8 | 135 | 85 |
| T2DM009 | Female | 62 | 158 | 67 | 26.8 | 150 | 95 |
| T2DM010 | Female | 50 | 160 | 82 | 32 | 120 | 80 |
| T2DM011 | Female | 59 | 165 | 72 | 26.4 | 140 | 85 |
| T2DM012 | Male | 36 | 175 | 78 | 25.5 | 160 | 90 |
| T2DM013 | Female | 65 | 158 | 70 | 28 | 165 | 95 |
| T2DM014 | Female | 42 | 160 | 65 | 25.4 | 130 | 85 |
| T2DM015 | Male | 43 | 180 | 85 | 26.2 | 125 | 80 |
| T2DM016 | Male | 75 | 170 | 90 | 31.1 | 120 | 80 |
| T2DM017 | Male | 40 | 172 | 75 | 25.4 | 145 | 90 |
| T2DM018 | Male | 40 | 170 | 80 | 27.7 | 130 | 85 |
| T2DM019 | Female | 43 | 160 | 70 | 27.3 | 120 | 80 |
| T2DM020 | Female | 62 | 162 | 78 | 29.7 | 130 | 85 |
| T2DM021 | Male | 63 | 168 | 85 | 30.1 | 140 | 85 |
| T2DM022 | Male | 60 | 170 | 82 | 28.4 | 150 | 90 |
| T2DM023 | Female | 40 | 160 | 66 | 25.8 | 145 | 90 |
| T2DM024 | Male | 32 | 175 | 78 | 25.5 | 125 | 80 |
| T2DM025 | Male | 50 | 172 | 80 | 27 | 135 | 85 |
| T2DM026 | Male | 71 | 170 | 90 | 31.1 | 120 | 80 |
| T2DM027 | Female | 73 | 155 | 72 | 30 | 130 | 85 |
| T2DM028 | Male | 60 | 180 | 85 | 26.2 | 130 | 85 |
| T2DM029 | Male | 65 | 168 | 82 | 29.1 | 140 | 90 |
| T2DM030 | Male | 33 | 175 | 80 | 26.1 | 130 | 85 |

# Master chart for biochemical parameters and UCP2 genotype of T2DM patients

| Sample I.D. | HbA1c | Total Cholesterol | TGL | HDL | LDL | VLDL | FBS | UCP2 genotype |
|---|---|---|---|---|---|---|---|---|
| T2DM001 | 6.7 | 85 | 99.2 | 30 | 35.16 | 19.84 | 113 | GG |
| T2DM002 | 6.6 | 120 | 193 | 35 | 46.4 | 38.6 | 112 | GA |
| T2DM003 | 7.4 | 75 | 87.7 | 32 | 25.46 | 17.54 | 108.9 | AA |
| T2DM004 | 6.7 | 193 | 147.1 | 51.1 | 112.48 | 29.42 | 108.1 | GG |
| T2DM005 | 6.1 | 159 | 145.3 | 40.7 | 89.24 | 29.06 | 121.6 | GA |
| T2DM006 | 6.7 | 112 | 89.6 | 38 | 56.08 | 17.92 | 112.3 | GA |
| T2DM007 | 9 | 123 | 120.6 | 38 | 60.88 | 24.12 | 147 | AA |
| T2DM008 | 6.5 | 144 | 116.8 | 35 | 85.64 | 23.36 | 134.6 | GG |
| T2DM009 | 11.6 | 110 | 241.5 | 38 | 23.7 | 48.3 | 268.4 | GG |
| T2DM010 | 6.3 | 105 | 184.8 | 38 | 30.04 | 36.96 | 106.3 | GA |
| T2DM011 | 7.7 | 168 | 134.7 | 40.1 | 100.96 | 26.94 | 157.7 | GA |
| T2DM012 | 9.5 | 173 | 404.3 | 40 | 52.14 | 80.86 | 230.2 | AA |
| T2DM013 | 13.3 | 127 | 140.1 | 38 | 60.98 | 28.02 | 403 | GG |
| T2DM014 | 6.5 | 120 | 115.6 | 38 | 58.88 | 23.12 | 128.4 | GG |
| T2DM015 | 6.7 | 205 | 132.1 | 38 | 140.58 | 26.42 | 124.2 | AA |
| T2DM016 | 6.5 | 85 | 69.4 | 35 | 36.12 | 13.88 | 118.7 | AA |
| T2DM017 | 9.7 | 130 | 247 | 50.5 | 30.1 | 49.4 | 158.2 | GG |
| T2DM018 | 6.7 | 130 | 158.7 | 43 | 55.26 | 31.74 | 110.4 | GA |
| T2DM019 | 6.5 | 132 | 231.3 | 41.8 | 43.94 | 46.26 | 105.5 | GG |
| T2DM020 | 6.1 | 163 | 126.1 | 44.5 | 93.28 | 25.22 | 120.2 | AA |
| T2DM021 | 7.7 | 98 | 112.9 | 35 | 40.42 | 22.58 | 212.2 | GG |
| T2DM022 | 7.8 | 144 | 194.7 | 36 | 69.06 | 38.94 | 175.7 | GA |
| T2DM023 | 9.8 | 216 | 191.9 | 42 | 135.62 | 38.38 | 239.9 | AA |
| T2DM024 | 8 | 132 | 82.5 | 35 | 80.5 | 16.5 | 156.9 | AA |
| T2DM025 | 7.3 | 262 | 191.3 | 40 | 183.74 | 38.16 | 170.2 | GA |
| T2DM026 | 6.5 | 115 | 109 | 35 | 58.2 | 21.8 | 103.1 | GA |
| T2DM027 | 8.4 | 115 | 73.8 | 42 | 58.24 | 14.76 | 77.3 | AA |
| T2DM028 | 6.4 | 95 | 133 | 29.4 | 39 | 26.6 | 121.9 | GG |
| T2DM029 | 7.1 | 224 | 200 | 38 | 154 | 31 | 157 | GG |
| T2DM030 | 6.5 | 98 | 78.4 | 35 | 48.12 | 14.88 | 138.1 | GA |

# Master chart for demographic parameters of healthy controls

| Sample I.D | Gender | Age | Height (cm) | Weight (kg) | BMI | SBP | DBP |
|---|---|---|---|---|---|---|---|
| C001 | Male | 43 | 175 | 80 | 26.1 | 120 | 80 |
| C002 | Male | 37 | 180 | 90 | 27.8 | 130 | 85 |
| C003 | Female | 42 | 165 | 68 | 24.98 | 110 | 75 |
| C004 | Male | 50 | 170 | 85 | 29.41 | 140 | 90 |
| C005 | Male | 57 | 175 | 95 | 31.02 | 135 | 85 |
| C006 | Female | 33 | 160 | 55 | 21.48 | 115 | 75 |
| C007 | Male | 33 | 185 | 85 | 24.8 | 125 | 80 |
| C008 | Male | 61 | 170 | 100 | 34.6 | 140 | 90 |
| C009 | Male | 35 | 175 | 80 | 26.12 | 120 | 80 |
| C010 | Female | 51 | 160 | 70 | 27.34 | 115 | 80 |
| C011 | Female | 44 | 155 | 65 | 27.2 | 110 | 75 |
| C012 | Female | 69 | 160 | 72 | 28.13 | 130 | 85 |
| C013 | Male | 33 | 180 | 80 | 24.69 | 125 | 85 |
| C014 | Female | 32 | 165 | 70 | 25.71 | 115 | 80 |
| C015 | Male | 60 | 175 | 90 | 29.39 | 140 | 90 |
| C016 | Female | 56 | 160 | 65 | 25.39 | 130 | 85 |
| C017 | Male | 44 | 175 | 85 | 27.76 | 130 | 90 |
| C018 | Male | 38 | 180 | 85 | 26.23 | 125 | 85 |
| C019 | Female | 48 | 160 | 70 | 27.34 | 120 | 80 |
| C020 | Male | 31 | 175 | 80 | 26.12 | 115 | 75 |
| C021 | Female | 58 | 165 | 75 | 27.55 | 130 | 85 |
| C022 | Female | 30 | 155 | 60 | 24.97 | 110 | 70 |
| C023 | Male | 60 | 175 | 85 | 27.76 | 125 | 80 |
| C024 | Male | 60 | 170 | 80 | 27.68 | 130 | 85 |
| C025 | Female | 30 | 160 | 55 | 21.48 | 110 | 75 |
| C026 | Male | 56 | 175 | 85 | 27.76 | 130 | 85 |
| C027 | Male | 65 | 160 | 70 | 27.34 | 120 | 80 |
| C028 | Male | 35 | 175 | 80 | 26.12 | 125 | 80 |
| C029 | Male | 38 | 180 | 85 | 26.23 | 120 | 80 |
| C030 | Male | 39 | 175 | 85 | 27.76 | 125 | 80 |

# Master chart for biochemical parameters and UCP2 genotype of healthy controls

| Sample I.D | HbA1c | Total Cholesterol | TGL | HDL | LDL | VLDL | FBS | UCP2 Genotype |
|---|---|---|---|---|---|---|---|---|
| C001 | 5.7 | 95 | 111.3 | 40 | 32.74 | 22.26 | 94.9 | GG |
| C002 | 5.7 | 130 | 195.6 | 40.7 | 50.18 | 39.12 | 84 | GG |
| C003 | 5.1 | 115 | 178.5 | 48.9 | 30.4 | 35.7 | 95.9 | GA |
| C004 | 5.5 | 159 | 152.2 | 40 | 88.56 | 30.44 | 89.9 | GA |
| C005 | 5.5 | 180 | 154.2 | 36 | 113.16 | 30.84 | 86.9 | AA |
| C006 | 5.3 | 140 | 72 | 54.1 | 71.5 | 14.4 | 82.9 | GG |
| C007 | 5.1 | 146 | 209.1 | 47.6 | 56.58 | 41.82 | 91.9 | GA |
| C008 | 5.7 | 203 | 287.4 | 40 | 105.52 | 57.48 | 85.1 | GA |
| C009 | 5.7 | 131 | 124.6 | 38.5 | 67.58 | 24.92 | 94.9 | GG |
| C010 | 5.7 | 201 | 356.8 | 40 | 89.64 | 71.36 | 95.8 | GA |
| C011 | 5 | 215 | 101.8 | 49.5 | 145.14 | 20.36 | 90.3 | GG |
| C012 | 5 | 133 | 148.7 | 38 | 65.26 | 29.74 | 107.5 | GG |
| C013 | 5.2 | 167 | 101.2 | 35 | 111.76 | 20.24 | 85.2 | GA |
| C014 | 5.3 | 179 | 78.7 | 40.9 | 122.36 | 15.74 | 86.1 | GA |
| C015 | 5.2 | 156 | 158.3 | 36 | 88.34 | 31.66 | 116.6 | AA |
| C016 | 5.7 | 238 | 172.7 | 40 | 163.46 | 34.54 | 89.7 | AA |
| C017 | 5.3 | 210 | 198.6 | 42 | 128.28 | 39.72 | 102.1 | GA |
| C018 | 5.3 | 190 | 170.3 | 40 | 150.63 | 37.38 | 95.4 | GG |
| C019 | 5 | 125 | 141.8 | 38 | 58.64 | 28.36 | 103.2 | GG |
| C020 | 5 | 162 | 155.6 | 38 | 92.88 | 31.12 | 78.6 | GA |
| C021 | 5.5 | 203 | 125.3 | 42.3 | 135.64 | 25.06 | 110.7 | GA |
| C022 | 5 | 201 | 82.6 | 44.2 | 140.28 | 16.52 | 85.4 | AA |
| C023 | 5.2 | 150 | 78.5 | 36 | 98.3 | 15.7 | 83.6 | GG |
| C024 | 5.6 | 115 | 70.9 | 36 | 64.82 | 14.18 | 94.1 | GA |
| C025 | 5.2 | 149 | 89.7 | 40 | 91.06 | 17.94 | 83.5 | AA |
| C026 | 5.5 | 138 | 85.7 | 40 | 80.86 | 17.14 | 90.7 | GG |
| C027 | 5.1 | 105 | 62.6 | 34.6 | 57.88 | 12.52 | 87.6 | GA |
| C028 | 5.1 | 105 | 82.4 | 39.5 | 49.02 | 16.48 | 96.9 | AA |
| C029 | 5.2 | 130 | 179.1 | 28.8 | 65.38 | 35.82 | 86 | GG |
| C030 | 5.1 | 198 | 164.9 | 38 | 127.02 | 32.98 | 91.9 | GA |